 DOI: 10.18231/2394-5478.2017.0049

Comparative evaluation of two direct microscopic methods in rapid diagnosis of superficial fungal infection

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Abstract

Introduction: Dermatomycosis is a common fungal infection seen in tropical and subtropical countries. Laboratory diagnosis of Dermatomycosis like dermatophytosis and Pityriasis versicolor depends on the demonstration of the etiological agents by microscopy and isolation of the fungi by culture. Culture on SDA is considered as gold standard however it is time consuming, so microscopic methods are used as rapid diagnostic tool.

Aims: Study was aimed at comparing two microscopic methods, KOH and Calcofluor white in the diagnosis of superficial fungal infections.

Methodology: Hair, nail and skin scrapings from patients who were clinically suspected with superficial fungal infections were divided into three parts and subjected to two direct microscopic methods (KOH and Calcofluor white) and culture.

Results: A total of 100 patients with superficial fungal infections were studied. 72 of them were clinically suspected cases of dermatophytosis and 28 of them were suspected cases of Pityriasis versicolor. Out of 100 samples, maximum number of samples were skin samples (84%) followed by nail (10%) and Hair (6%). Among the skin samples T.corporis (31%) was the predominant clinical manifestation followed by T.versicolor (28%). 28 cases of Tinea versicolor were not cultured. Out of 72 samples which were cultured, 29(40.27%) were culture positive and 43(59.7%) were culture negative, 32 were KOH positive whereas 39 samples were positive by CFW. Among culture positive samples T. rubrum was predominant isolate grown. Out of 100 samples 49 samples were KOH positive and 58 samples were Calcofluor white positive.

Conclusion: KOH wet mount using bright field microscopy is quick, inexpensive and routinely used for diagnosing fungal infections, but it does not produce a colour contrast and requires skill to interpret. While CFW provides a colour contrast and the diagnosis is easier, faster as present study also shows 10% of samples were CFW positive were missed by KOH examination.

Keywords: Calcofluor white, Dermatophytosis, Potassium hydroxide, Superficial mycosis.

Introduction

Dermatomycosis is a common fungal infection seen in tropical and subtropical countries affecting the skin and its appendages. The 3 major genera of dermatophytes which cause infection are Trichophyton, Epidermophyton and Microsporon[(1)] with more than 200 species in each. Other common superficial fungal infection is Pityriasis versicolor.

Laboratory diagnosis of superficial fungal infections like dermatophytosis and Pityriasis versicolor depends on the demonstration of the etiological agents by microscopy and isolation of the fungi by culture.

Isolation of the etiological agent by culture on SDA is considered as gold standard for the diagnosis of fungal infections as it is highly specific and also enables us to identify and speciate the isolate, the major drawback of fungal culture is that, it is time consuming[(2)] and thus, microscopic methods are used as rapid diagnostic tool.

There are various microscopic methods available for the diagnosis of fungal infections, but KOH is the most commonly used method in limited resource settings as it is cost effective. Many positive cases may be missed when only KOH is used as the microscopic method due to lack of contrast and low visibility and also due to and lack of skill of the observer.[(3)] The sensitivity of microscopy may be increased if a contrast is created as in calcofluor white, thus the study was aimed at comparing KOH and Calcofluor white as the microscopic methods in the diagnosis of superficial fungal infections.

Materials & Methods

Hair, nail and skin scrapings from patients who were clinically suspected with superficial fungal infections were included in this study. The affected part of the skin was cleaned with 70% alcohol and the active edge of the lesion was scrapped with a sterile blunt scalpel. The infected hair was removed by plucking the hair with the roots intact using epilating forceps. Nail clippings of the infected nail was taken by lifting the nail.

The samples thus obtained were divided into three parts and subjected to two direct microscopic methods and culture.

Direct Microscopic Methods

Potassium Hydroxide(KOH) Mount: A small piece of the sample was placed on a clean glass slide and 2-3 drops of 20-40% KOH was added and a cover slip was placed. The slide was incubated for about 30 minutes or until softening and digestion of the specimen occurred and then screened under 40X of the microscope for the presence of fungal elements.

Calcofluor White Staining(CFW): The second part of the sample was transferred to a clean glass slide and a drop of calcofluor white stain(Sigma-aldrich, Lot no-BCBK6948V, Rs- 2000) with 1 drop of 10% KOH was added to the sample. A cover slip was placed over the slide and it was allowed to stand for 1minute. The slide was observed under fluorescent microscope using blue light excitation for the presence of fungal elements, which appeared bright green to blue, while other materials appeared reddish orange.

The third part of the sample was used for culture and was inoculated on to SDA with chloramphenicol and cyclohexidene and the culture isolate was identified by observing the microscopic morphology like organization of hyphae, presence of micro and macroconidia.

Results

A total of 100 patients with superficial fungal infections were studied. 72 of them were clinically suspected cases of dermatophytosis and 28 of them were suspected cases of Pityriasis versicolor.

Our study showed that the maximum number of patients suspected of superficial fungal infections were in the age group of 21-40 years, followed by patients in the age group of 41-60 years and above. Patients in the age group of 0-20 years were least prone for infection.

In our study males were predominantly affected (67%) as compared to females (33%). Out of 100 samples, maximum number of samples were skin samples (84%) followed by nail (10%) and Hair (6%). Sex wise distribution of samples is shown in Table 1.

Table 1: Sex wise distribution of clinical samples

Samples (100)	Males (67) 67%	Females (33) 33%
Skin- 84	58(59%)	26(26%)
Hair-06	4(4%)	2(2%)
Nail-10	5(5%)	5(5%)

Among the skin samples T.corporis (31%) was the predominant clinical manifestation followed by T.versicolor (28%). Microscopic examination of skin samples from suspected Pityriasis versicolor showed maximum positive results with CFW stain. Results of KOH and CFW stain in diagnosis of Pityriasis versicolor is shown in Table 2. Out of total 100 samples examined by microscopic methods 09 samples were CFW positive but negative by KOH examination. Results of KOH and CFW are shown in Table 3.

Table 2: KOH & CFW Findings in the diagnosis of pityriasis versicolor

Total	KOH positive	KOH negative	CFW positive	CFW negative
28	17(60.7%)	11(39.2%)	19(67.8%)	9(32.1%)

Table 3: KOH & CFW Findings in the present study

Microscopy method	CFW positive	CFW negative	Total
KOH positive	49	00	49
KOH negative	09	42	51
Total	58	42	100

Of the 100 samples processed 28 were cases of Tinea versicolor and were not cultured. Out of 72 samples which were cultured, 29(40.27%) were culture positive, 43(59.7%) were culture negative, 32(44.4%) were KOH positive and 39(54.1%) were CFW positive. Comparative results of culture, KOH and CFW are shown in Table 4.

Table 4: Comparison of results of culture, KOH and CFW

Total Cases	Culture Positive	KOH Positive	CFW Positive
72	29 (40.27%)	32 (44.4%)	39 (54.1%)

Among 29 culture positive samples Trichophyton rubrum was grown in 22(75.86%) samples followed by Trichophyton mentagrophytes in 05(17.24%) and Trichophyton violaceum in 02(6.89%) samples. Microsporum and Epidermophyton species were not isolated from any samples in our study. Growth of different dermatophytes from various types of clinical types is shown in Table 5.

Table 5: Different Clinical types and different types of dermatophytes growth

Clinical types	T. rubrum	T.mentagrophyte	T. violaceum	Total
T.corporis	12	04	01	17
T.capitis	03	00	01	04
T.unguium	01	00	00	01
T.cruris	03	00	00	03
T.capitis	03	00	00	03
T.pedis	00	01	00	01
Total	22	05	02	29

Discussion

Direct microscopy by KOH and fungal culture are the common methods used in diagnosis of dermatophytosis. KOH preparation is simplest and most inexpensive method for direct microscopy. However it has been reported to have false negative rate of 5% to 15%, possibly because of low visibility of scantily scattered fungal material.[4]

Fungal culture remains the gold standard; however false negative culture findings may arise when sample contains only dead or non-viable fungal organisms probably after treatment, thus, a positive microscopy is of major importance to confirm the diagnosis and also to continue the treatment. CFW is a fluorochrome which can be used as an alternative to KOH to improve detection of fungi in clinical samples.

In our study, maximum numbers of cases of superficial fungal infection was seen in the age group of 21-40 years (49%), followed by 41-60 years (31%), this is in accordance with the study conducted by Anasuya devi et al[5] and Suruchi Bhagra et al,[6] who reports a peak incidence of infection in 21-40 years age group 41% and 52% respectively. Similar peak in this age group has been reported by Ravi et al[7] and Mohanty J C et al.[8] It is also observed in our study that males are more commonly affected (67%) than females (33%) with a male to female ratio being 2:1; this is also in accordance to many researchers.[5,6,7,8] The higher incidence in males and in the 20-40 age groups may be attributed to vigorous outdoor activity with increased perspiration which predisposes to tinea infection.

In present study T.corporis (31%) was the most common presentation followed by T.unguium (10%) and T.cruris (8%), 28% of the samples received were found to be due to T.versicolor infection. Suruchi et al.[6] also reports T.corporis as the commonest clinical entity (27.27%). Similar predominance of T.corporis has been reported by Singh & Beena[9] followed by T.cruris (12.3%). Kanwar A J et al,[10] Sanjeewani Fonseka et al.[11] reports T.versicolor infection to be 36% and we report 28% of superficial infections to be due to T.versicolor.

Of the 100 cases studied, KOH was positive in 49 and microscopy by CFW staining was positive in 58, that is, we were able to detect 9 more cases of superficial fungal infection by using CFW as the microscopic method. Of the 100 cases suspected of superficial fungal infection, 28 cases were of T.versicolor and among these 28 cases; KOH was positive in 60.7% and CFW was positive in 67.8% of samples, we were able to detect 2 more cases of T.versicolor by using CFW as microscopic method.

Of the 72 samples which were cultured, 29 (49.27%) samples were culture positive, while KOH and CFW were positive in 32 and 39 samples respectively. All the samples which were culture positive were also positive by both the microscopic methods. 10 samples which were culture negative were CFW positive and 3 samples which were culture negative were KOH positive. The false negativity of culture might be due to dead or non-viable fungal elements due to treatment or sample used for culture inoculation may not have contained the fungal elements. Similar findings have been seen in other studies.[12,13,14] Among 29 culture positive samples Trichophyton rubrum(22) was predominant isolate grown followed by Trichophyton mentragrophyte(05). Similar observations were also reported by Suruchi Bhagra et al.[6]

Direct microscopy plays an important role in diagnosis of fungal infections; however culture gives a definitive diagnosis. Of the culture negative cases, 10 showed fungal elements by CFW staining but failed to grow in culture, this could be due to non-viability of fungi prior to inoculation.

When compared to culture, 13.83% of samples were positive by CFW, however, the difference between culture positivity and KOH was only 4.13%. Direct microscopy with CFW is found to be more sensitive than KOH and culture in detecting fungal etiology in the present study. In resource poor settings, KOH can still be used as the microscopic method for diagnosis of superficial fungal infection as it is cheap and simple method. CFW stain even though superior to KOH and culture, requires fluorescent microscope and skilled personnel.

Conclusion

Although Dermatophytosis is not a life threatening disease, complications do occur. Definite diagnosis is necessary to provide appropriate treatment to the patient. KOH wet mount using bright field microscopy is quick, inexpensive and routinely used for diagnosing fungal infections, but it does not produce a colour contrast and requires skill to interpret. While CFW provides a colour contrast and the diagnosis is easier, faster. In our study, we have found 10% of cases to be positive by CFW which were missed by KOH.

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