International Journal of Research in Dermatology
Bose AK et al. Int J Res Dermatol. 2022 Jul;8(4):388-391
http://www.ijord.com

DOI: https://dx.doi.org/10.18203/issn.2455-4529.IntJResDermatol20221635

Original Research Article

Diagnostic utility of Chicago sky blue stain in superficial dermatophytosis

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Received: 28 May 2022
Accepted: 13 June 2022

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ABSTRACT

Background: Dermatophyte infections are one of the commonest presentations to a dermatology OPD. The clinical lesions are highly variable and closely resemble other skin diseases, thus it is important to confirm one's diagnosis using rapid, cheap methods.
Methods: Skin scrapings from the lesions of 352 suspected cases of dermatophytosis were subjected to KOH mount and KOH with Chicago sky blue (CSB) stain for direct microscopic examination and culture using Sabouraud's dextrose agar (SDA). Taking the culture as the gold standard method for diagnosis, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of KOH mount and CSB staining method was calculated. Kappa statistics were used to determine the degree of congruence between the various diagnostic methods.
Results: Direct microscopy with CSB stain, KOH mount and mycological culture showed positive results in 251 (71.3%), 227 (64.5%) and 292 (82.9%) patients, respectively. Cohen's Kappa was used to see the agreement between KOH mount and culture (Kappa=0.09) which was statistically significant ($p<0.05$) at 95% CI. CSB staining and Culture (Kappa=0.09) also showed negligible agreement (Kappa=0.09) which was statistically significant ($p<0.05$). CSB staining and KOH mount showed substantial agreement (Kappa=0.8) which statistically significant ($p<0.05$).
Conclusions: The addition of CSB stain to KOH provides a colour contrast that highlights the fungal elements well, making interpretation easy which increases the overall diagnostic accuracy. CSB stain is inexpensive, easily available and provides a rapid method to diagnose dermatophytosis.

Keywords: CSB, Culture, Dermatophytosis, Potassium hydroxide mount

INTRODUCTION

Fungal skin infections are categorized into superficial and deep. Superficial infections are defined as those limited to the stratum corneum of the epidermis, or the hair and nails. The three most common types of superficial mycoses are dermatophytosis, diseases caused by Malassezia and superficial candidiasis.[1] The prevalence of dermatophytosis varies according to geographical location and climatic conditions. In the world, 20-25% of people are infected by dermatophytosis, while in India it ranges from 36.6-78.4%.[2,3] Although the majority of the studies are hospital-based, an increasing trend of dermatophytosis is evident in published literature. Dermatophytosis infection most commonly presents as blackish macules with scaling associated with itching and irritation. Though the infection is non-invasive and curable, its widespread nature and therapeutic costs are a major public health problem. The most economic and easiest method for diagnosis of dermatophytosis is by direct examinations of samples using a KOH preparation.[4] However, the gold standard for diagnosis of the infection is culture, which is rarely used as it is time-consuming and expensive. Also, the standard KOH

preparation wet mount lacks a colour contrast and depends on the skills of the observer for the detection of fungal elements. CSB 6B stain is a new promising contrast stain that can stain the fungal hyphae and spores with a blue contrast against a light purplish background of cellular debris making them easily identifiable under a light microscope. Only a few reports have been published on using CSB as a diagnostic modality for dermatophytosis, despite it being easily available. Thus, the current study was planned to explore the feasibility and reliability of CSB as a diagnostic modality for the diagnosis of superficial dermatophytosis.

METHODS

A cross-sectional, observational study was conducted in the dermatology OPD of a tertiary care hospital. Permission for conducting the study was obtained from the institutional ethics committee. A total of 352 suspected cases of superficial dermatophytosis were included in the study over 9 months. After obtaining informed consent for participation in the study, sociodemographic details of all patients were noted. Thereafter relevant laboratory investigations were carried out.

Scraping method

After cleaning the affected area with 70% isopropyl alcohol, scraping of skin was taken with the blunt edge of a sterile scalpel from the edge of the lesion and was placed on two clean microscopic slides. The 10% KOH was added to both microscopic slides. CSB was added to one of the slides and both were examined microscopically after 30 minutes. The slides were visualized under low power (10× magnification) as well as high power (40× magnification). The presence of hyphae and arthroconidia was considered positive for the presence of dermatophytes in both KOH and CSB stains.

Culture

The samples were collected in a sterile container. Scales were inoculated on SDA and dermatophyte test medium (DTM). Culture medium incubated at 300ºC in a BOD incubator. Growth of dermatophytes was identified by colony morphology and other standard mycological tests.

Data were entered using Microsoft excel and analysis was done using SPSS version 22. Taking culture method as reference method for diagnosis, sensitivity, specificity, PPV, NPV of KOH and CSB staining method calculated. Kappa statistics were used to determine the degree of congruence between the various diagnostic methods.

RESULTS

A total of 352 patients were included in the study. KOH mount showed 227 (64.5%) of the samples to be positive for fungal elements while others were negative. When CSB staining was added to KOH mount 251 (71.3%) samples were found to be positive for fungal elements. While culture showed growth of fungal species in 292 (82.9%) of the specimens. The distribution of species of dermatophytes identified in culture is shown in Table 1. The sensitivity, specificity, PPV, NPV of the KOH mount and CSB staining method taking mycological culture as the reference method is enumerated in Table 2. Cohens Kappa was used to see the agreement between KOH mount and culture which showed negligible agreement (Kappa=0.09) which was statistically significant ($p<0.05$) at 95% of CI (Table 3). KOH with CSB staining and culture (Kappa=0.09) also showed negligible agreement (Kappa=0.09) which was statistically significant ($p<0.05$) (Table 4). KOH with CSB staining and KOH mount showed substantial agreement (Kappa-0.8) which was statistically significant ($p<0.05$) (Table 5).

Table 1: Distribution of dermatophyte species.

Fungal species	Frequency	Percentage (%)
Aspergillus niger	4	1.1
Candida albicans	21	6
Candida glabrata	4	1.1
T. mentagrophytes, C. parapsilosis	4	1.1
C. parapsilosis	21	6
T. rubrum	101	28.7
T. mentagrophytes	129	36.6
T. tonsurans	4	1.1
Trichosporum	4	1.1
No growth	60	17
Total	352	100

Table 2: Comparison of diagnostic accuracy of CSB stain and KOH (culture as reference).

Parameters	KOH mount	KOH+CSB
Sensitivity	69.57	98.32
Specificity	38.89	46.45
PPV	73.6	99.3
NPV	34.29	43.29
Diagnostic accuracy	63.22	86.28

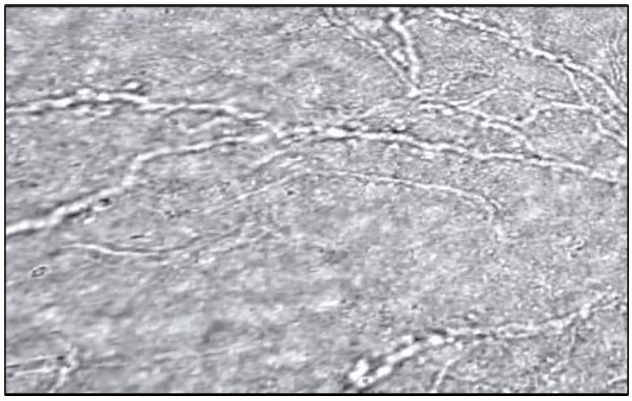

Figure 1: The 10% KOH mount of dermatophytes with refractile, septate and branching hyphae.

Table 3: Culture versus KOH mount in the diagnosis of superficial dermatophytosis (test for agreement).

Culture	Slide KOH		Total	Kappa value#	P value*	Degree of agreement (%)
	FES	NFE				
FES	195	97	292	0.094	0.047	57.00
	66.80%	33.20%	100%			
NFE	32	28	60			
	53.30%	46.70%	100%			
Total	227	125	352			
	64.50%	35.50%	100%			

*P<0.05=significant at 95% CI. #Kappa interpretation: <0: poor agreement, 0.0-0.20: slight agreement, 0.21-0.40: fair agreement, 0.41-0.60: moderate agreement, 0.61-0.80: substantial agreement, 0.81-1.00: almost perfect agreement.

Table 4: Culture versus CSB staining in diagnosis of superficial dermatophytosis (test for agreement).

Culture	Slide KOH+CSB		Total	Kappa value#	P value*	Degree of agreement (%)
	FES	NFE				
FES	211	81	292	0.094	0.047	51.3
	72.30%	27.70%	100.00%			
NFE	40	20	60			
	66.70%	33.30%	100.00%			
Total	251	101	352			
	71.30%	28.70%	100.00%			

*P<0.05=Significant at 95% CI, #Kappa interpretation: <0: poor agreement, 0.0-0.20: slight agreement, 0.21-0.40: fair agreement, 0.41-0.60: moderate agreement, 0.61-0.80: substantial agreement, 0.81-1.00: almost perfect agreement.

Table 5: KOH plus CSB staining versus KOH mount in diagnosis of superficial dermatophytosis (test for agreement).

Slide KOH	Slide KOH+CSB		Total	Kappa value#	P value*	Degree of agreement (%)
	FES	NFE				
FES	223	4	227	0.793	0.00	0.93
	98.20%	1.80%	100.00%			
NFE	28	97	125			
	22.40%	77.60%	100.00%			
Total	251	101	352			
	71.30%	28.70%	100.00%			

*P<0.05=Significant at 95% CI, #Kappa interpretation: <0: poor agreement, 0.0-0.20: slight agreement, 0.21-0.40: fair agreement, 0.41-0.60: moderate agreement, 0.61-0.80: substantial agreement, 0.81-1.00: almost perfect agreement.

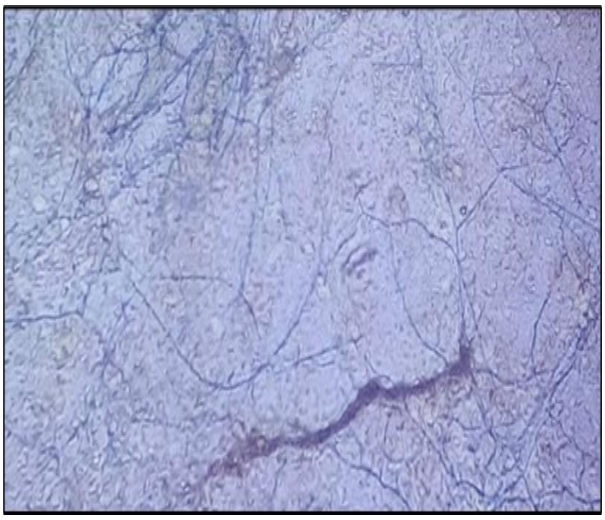

Figure 2: The 10% KOH with CSB mount of dermatophytes with branching and septate hyphae.

DISCUSSION

We observed that *T. mentagrophytes* is the most commonly identified species followed by *T. rubrum*. The least commonly identified species was *Aspergillus niger*. Nenoff et al similarly in their study reported *T. mentagrophytes* and *T. rubrum* as the most commonly identified species of dermatophytosis.[9] Also, Verma et al in their systemic review stressed the increasing prevalence of *T. mentagrophytes* and *T. rubrum* from studies around the world.[6] Our study found that although combining CSB staining with KOH mount increases the sensitivity of diagnosis of superficial dermatophytosis as compared to only KOH, also the specificity, PPV and NPV increases. The diagnostic accuracy of KOH with CSB increased to 98.32% compared to only 69.57% in the case of culture. In congruence with our findings, Lodha et al demonstrated 100% sensitivity, high specificity, PPV and NPV for KOH plus CSB in the diagnosis of superficial dermatophytes.[4] Also, Lim et al

and Tambiosis et al reported a better sensitivity and specificity for the diagnosis of superficial dermatophytosis on using CSB stain as a contrast.[10,11] We observed negligible agreement between KOH wet mount and culture while a very high agreement was observed between KOH wet mount and KOH with CSB staining in the diagnosis of superficial dermatophytosis. This was a unique finding in our study as very few studies in the open domain have tried to establish an agreement between the new diagnostic methods with an already established method of diagnosis for superficial dermatophytosis.

CONCLUSION

In conclusion, the addition of CSB stain to KOH provides a colour contrast that highlights the fungal elements well, making interpretation easy. This increases the overall diagnostic accuracy of it as compared to established methods of diagnosis. Also, CSB stain is inexpensive and requires an ordinary light microscope for reading as opposed to other methods which are technically demanding and expensive. Further population-based studies involving a larger sample size should be undertaken to mainstream KOH plus CSB as a method of diagnosis of dermatophytosis which would help in early diagnosis and treatment initiation. This would go a long way in halting the silent epidemic of dermatophytosis in the world.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Superficial Mycoses: Dermatophytosis. Infectious Disease Advisor. 2019. Available at: https://www.infectiousdiseaseadvisor.com/home/decision-support-in-medicine/infectious-diseases/superficial-mycoses-dermatophytosis/. Accessed on 2021 Nov 26.
2. Kalita J, Goyal G, Kumar P, Misra UK. Intracerebral hemorrhage in young patients from a tertiary neurology center in North India. J Neurol Sci. 2014;336(1-2):42-7.
3. Kalita JM, Sharma A, Bhardwaj A, Nag VL. Dermatophytoses and spectrum of dermatophytes in patients attending a teaching hospital in Western Rajasthan, India. J Fam Med Prim Care. 2019;8(4):1418-21.
4. Poojary S, Lodha N. A novel contrast stain for the rapid diagnosis of pityriasis versicolor: A comparison of Chicago Sky Blue 6B stain, potassium hydroxide mount and culture. Indian J Dermatol. 2015;60(4):340.
5. Sentamilselvi G, Kamalam A, Ajithadas K, Janaki C, Thambiah AS. Scenario of chronic dermatophytosis: an Indian study. Mycopathologia. 1998;140(3):129-35.
6. Verma SB, Panda S, Nenoff P, Singal A, Rudramurthy SM, Uhrlass S, et al. The unprecedented epidemic-like scenario of dermatophytosis in India: I. Epidemiology, risk factors and clinical features. Indian J Dermatol Venereol Leprol. 2021;87(2):154-75.
7. Hanumanthappa H, Sarojini K, Shilpashree P, Muddapur SB. Clinicomycological study of 150 cases of dermatophytosis in a tertiary care hospital in South India. Indian J Dermatol. 2012;57(4):322-3.
8. Gandhi S, Patil S, Patil S, Badad A. Clinicoepidemiological study of dermatophyte infections in pediatric age group at a tertiary hospital in Karnataka. Indian J Paediatr Dermatol. 2019;20(1):52.
9. Nenoff P, Verma SB, Vasani R, Burmester A, Hipler U-C, Wittig F, et al. The current Indian epidemic of superficial dermatophytosis due to Trichophyton mentagrophytes-A molecular study. Mycoses. 2019;62(4):336-56.
10. Lim S-L, Lim CS-H. New contrast stain for the rapid diagnosis of pityriasis versicolor. Arch Dermatol. 2008;144(8):1058-9.
11. Tambosis E, Lim C. A comparison of the contrast stains, Chicago blue, chlorazole black, and Parker ink, for the rapid diagnosis of skin and nail infections. Int J Dermatol. 2012;51(8):935-8.

Cite this article as: Bose AK, Kharkar V, Wanjare S, Nataraj G, Thirumalaiswamy A. Diagnostic utility of Chicago sky blue stain in superficial dermatophytosis. Int J Res Dermatol 2022;8:388-91.