Original Article

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Microbiology Section

Utility of Chicago Sky Blue Stain in Rapid Diagnosis of Mycotic Infections

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ABSTRACT

Introduction: Diagnosis of fungal infection is mostly relied on clinical skills assisted by array of diagnostic methods. Culture and molecular testing are specific but lacks sensitivity and are labour intensive, time consuming, expensive and not readily available. Direct examination using Potassium Hydroxide (KOH) mount is most often practiced but lacks contrast and requires a skilled eye to interpret. Chicago Sky Blue 6B (CSB) stain has shown a promising result providing coloured contrast and better sensitivity and specificity.

Aim: To evaluate the rapid diagnosis of fungal infections using CSB stain over conventional KOH mounts.

Materials and Methods: This cross-sectional analytical study was done at RIMS, Raipur catering to rural and tribal population in Chhattisgarh in Central India over a period of 10 months after due approval from Institutional Ethics Committee. Clinically, suspected cases of mycosis belonging to all ages of either gender attending Outpatient Department (OPD) of Dermatology and Ophthalmology Department, who were advised mycological evaluation by physician but not on treatment were included in study. The samples were processed with KOH mount and CSB stain and were observed by two independent observers. The data was managed in Microsoft office Excel and was analysed using tests of proportion and Pearson's Chi-square test for significance.

Results: A total of 128 participants were involved in the study with a male preponderance of 96/128 (75%) as against 32/128 (25%). A total of 110 patients were diagnosed using CSB 6B stain whereas 80 patients were diagnosed using KOH mount. CSB 6B was evaluated against the conventional KOH mount using a Chi-square test and was found to be highly significant with Chi-square value of 39.74 with 1 degree of freedom and p-value of <0.001.

Conclusion: This study brought out the significance of CSB 6B stain over conventional KOH mount with better sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) and ease and rapidity of diagnosis with better colour contrast helping clinicians with prompt initiation of treatment with a laboratory confirmation.

Keywords: Dermatophytosis, Keratitis, Potassium hydroxide mount

INTRODUCTION

The diagnosis of mycotic infections like Dermatophytic infections of skin, nails and hairs, keratitis, keratoconjunctivitis is usually clinical with laboratory methods like direct examination, culture and molecular techniques to assist the clinician. Most of the culture techniques are time consuming have low sensitivity whereas and molecular diagnostic methods are expensive as well as not readily available [1].

KOH mount of the lesion is easy and cheap way of confirming the diagnosis but it lacks contrast and requires skill to interpret. It is less sensitive with upto15% false negativity [2]. In a busy clinic, it is always helpful to have a rapid reliable and easy to interpret diagnostic technique. This helps in prompt initiation of correct treatment [1,3].

Rapid methods using contrast stains like parker blue-black ink and calcofluor white, have been used in the diagnosis. Parker Blue/Black ink has a disadvantage of a bluish precipitate and also doesn't work well with KOH. On the other hand, calcofluor white requires a fluorescent microscope for observation and thus is expensive [4].

CSB 6B stain along with 8% KOH as clearing agent is a new contrast stain that has shown promising results as a rapid and reliable diagnostic method for dermatophytosis [2,3]. It highlights the fungal hyphae and spores, blue against a purplish background and can be used with the routine bright field microscopes with good sensitivity and specificity [1,3].

Thus, this study was undertaken to evaluate the utility of CSB stain in direct microscopy against the conventional KOH mount for rapid diagnosis of mycotic infections.

MATERIALS AND METHODS

This cross-sectional prospective analytical study was carried out in the Department of Microbiology, Raipur Institute of Medical Sciences (RIMS), Raipur catering to rural and tribal population in Chhattisgarh in Central India over a period of 10 months from August 2019 to May 2020 after due approval from Institutional Ethics Committee (RIMS/ADMIN/261-H/2019 Dated 11.07.2019) with waiver of consent for using the biological sample sent for conventional KOH mount for further evaluation with CSB 6B stain.

A convenient sampling method was used wherein all clinically suspected cases of mycotic infections of irrespective of age and gender attending to OPD of Dermatology and Ophthalmology Department of RIMS, Raipur over the study period, who were advised mycological evaluation by direct microscopy by the treating physician were enrolled in the study whereas all patients already on the self or advised treatment were excluded. Clinically, suspected cases were characterised by skin lesions like spreading, scaly, crusty irregular or well demarcated area of erythema and alopecia or severe inflammatory suppurative lesions, moist macerated lesions or onychomycosis showing white patches to brittle nails with masses under nailbed to complete breakdown of nails. Patients of keratitis usually presented with pain, congestion and decreased or blurred vison.

Collection of samples: All the non-repetitive skin, nail and corneal scrapping samples from enrolled patients were collected over the study period. The skin scrapings were collected with blunt edge of scalpel from the suspected lesions after wiping the area with 70% ethanol and were transported immediately in piece of black paper sheet to mycology laboratory of Microbiology Department. In case

of affected nail, the nail was trimmed to the edge and the scrapping from underneath the nail was obtained or part of affected nail was cut and transported similarly. In case of ocular keratitis, corneal scrapings were collected by the ophthalmologist and were sent to laboratory immediately.

All such samples received at the Mycology laboratory were processed using conventional KOH and CBS stain:

1. **KOH mount**- A drop of 20% KOH was added to adequate amount of sample received on a clean new glass slide, covered with the coverslip and sealed using colourless nail paint [1].
2. **CSB staining-** A drop of 1% CSB 6B and 8% KOH as the clearing agent was added to adequate amount of sample received on a clean new glass slide and covered with the coverslip and sealed using colourless nail paint [1,3].

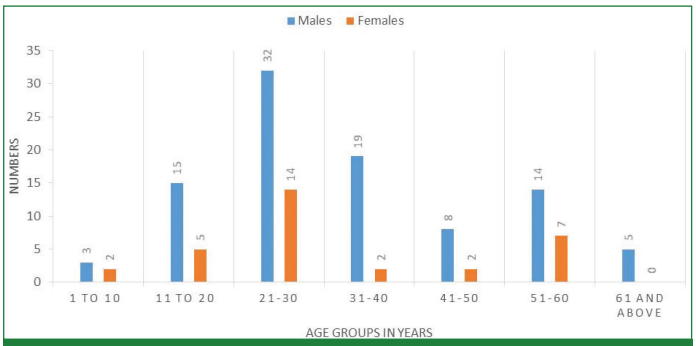
The prepared slides were left in humidified chamber for about 30 minutes and observed at 100x, 400x for screening and at 1000x magnification using oil immersion lens for better morphology by two independent observers involved in the study, to remove observer's bias. If enough keratinolysis was not achieved in 30 minutes, the slides were again observed after 8 hours. The observer's also noted the time taken to observe the slides, clarity in field of vision and contrast obtained for observing the fungal elements. The positive findings were matched and recorded, only if observations from both the observer were in unison.

STATISTICAL ANALYSIS

The data was managed in Microsoft Office Excel and was analysed using tests of proportion and Pearson's Chi-square test for significance. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) was also calculated against KOH mount being the most widely used method for rapid diagnosis of mycotic infections.

RESULTS

A total of 128 participants were involved in the study with a male preponderance of 96/128 (75%) as against females 32/128 (25%). The gender and age wise distribution of the participants in study group was as depicted in [Table/Fig-1].



[Table/Fig-1]: Gender and age wise distribution of participants.

Of the 128 participants involved in the study, 108 presented with the skin lesions and 15 presented with nail lesions whereas five patients were suspected cases of mycotic keratitis. Total number of patients with skin, nail and corneal involvement diagnosed using the different modalities are as depicted in [Table/Fig-2].

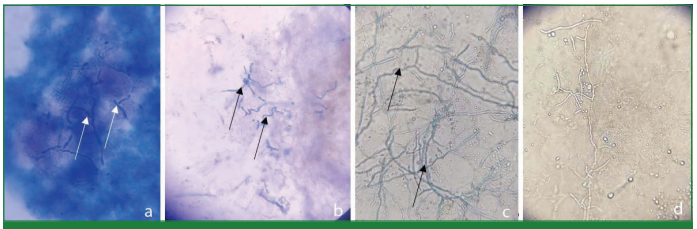
Mycotic infection presentation	Number of patients	Diagnosed using KOH mount	Diagnosed using CSB 6B
Skin lesion	108	73 (67.59%)	94 (87.03%)
Nail lesion	15	05 (33.33%)	12 (80%)
Keratitis	5	02 (40%)	04 (80%)
Total	128	80 (62.5%)	110 (85.93%)

[Table/Fig-2]: Number of patients diagnosed using KOH and CSB 6B stains in patients with different presentations.

A total of 110 patients were diagnosed using CSB 6B stain whereas 80 patients were diagnosed using KOH mount. Total number of patient's diagnosed gender wise is depicted in [Table/Fig-3]. A total of 30 more patients were diagnosed over KOH by using CSB stain which amounts to 23.43% more cases diagnosed as compared to conventional method of KOH mount. Both the observers reported clear fields with better contrast in case of CSB 6B stain [Table/Fig-4a-d].

Method used	Male	Female	Total	Time taken for diagnosis Mean±SD (minutes)
Chicago Sky Blue 6B	92 (83.63%)	18 (16.36%)	110/128 (85.93%)	3.42±0.5
KOH	70 (87.5%)	10 (12.5%)	80/128 (62.50%)	5.30±0.4

[Table/Fig-3]: Shows gender wise distribution of participants diagnosed using Chicago Sky Blue (CSB) 6B stain and KOH mounts.



[Table/Fig-4]: a) Coloured fungal elements with CSB 6B in skin scrapping even with incomplete keratinolysis; b) Sparse coloured fungal elements in corneal scrapping clearly seen with good contrast with CSB 6B stain; c) Clear visibility with good contrast with clear fields in CSB 6B stain in Dermatophytosis; d) KOH mount in Dermatophytosis (lesser contrast then CSB stain).

CSB 6B was evaluated against the conventional KOH mount using a Chi-square Test and was found to be highly significant [Table/Fig-5].

	KOH Positive	KOH Negative	Total	Chi-square value and p-value
Chicago Sky Blue 6B Positive	74	36	110	39.74 with 1 degree of freedom p<0.001
Chicago Sky Blue 6B Negative	6	12	18	
Total	80	48	128	

[Table/Fig-5]: Showing comparative evaluation of CSB against KOH using Pearson's Chi-square test.
p<0.001 is considered to be highly significant

The CSB 6B stain was found to be having sensitivity of 92.5%, specificity of 58.33% and NPV and PPV of 78.72% and 82.35%, respectively with better contrast and clean fields for observation when evaluated against the conventional KOH mount used commonly for direct microscopy.

DISCUSSION

Mycotic infections especially dermatomycosis, onychomycosis and mycotic keratitis are common problem in India, especially in the rural and tribal belt doing farming and exposed to moist and humid conditions prevalent in central India. Rapid confirmation of the clinically suspected cases of mycotic infections is of prime importance in view of prompt initiation of treatment in patients coming in OPD.

Conventional method of using KOH is inexpensive but lacks in contrast and do require a reasonable amount of skills for interpretation of the KOH mounts prepared which may results in false negative results in case of sparse fungal elements and incomplete keratinolysis. Culture on the other hand is very specific method but is difficult, time consuming, costly and less sensitive. Various molecular methods like probes, in-situ hybridisation and polymerase chain reaction, restriction endonuclease analysis, though sensitive and specific, are technologically demanding, labour intensive, costly not easily available in developing countries. Mycotic keratitis poses a problem especially because of sparse fungal elements in very small amount of corneal scraping samples, requiring use of special stains and fluorescent microscopy [1].

CSB 6B stain as a new promising contrast stain on the horizon which provides an excellent colour contrast for diagnosis of fungal infection. CSB is a direct diazo dye compound used primarily in the textile industry for dyeing cotton. It has also been used as counter stain for background auto fluorescence in immunohistochemistry and to assess viability of spermatozoa in animal studies and for dye assisted macular surgery [1].

A 1% CSB together with 8% KOH as the clearing agent is used which stains the fungal hyphae and spores a contrast blue against a light purplish background of cellular debris with an added advantage over KOH that it outlines the morphological features of both the spores and the hyphae and enables detailed study of morphology under oil-immersion microscopy [5].

In this study, more cases were diagnosed using CSB 6B as against conventional KOH mount. This is in concordance with other study done by Prakash R et al., which has shown a positivity to the extent of 94.5% with CSB as compared to KOH and calcofluor white with 75.3% and 83.5% respectively [6]. Similar results were also obtained by Lim CS and Lim SL with sensitivity of 86% for CSB against 75% for KOH mount [7]. In a study carried out by Lodha N and Poojary SA, in Mumbai on rapid diagnosis of pityriasis versicolour, CSB has been found to be having sensitivity of 98% as against the 92% of KOH [4].

The results of CSB with conventional KOH mount was compared and found to be highly significant as highly significant with chi-square Value of 39.74 with 1 degree of freedom and p-value of <0.001. These results were in concordance with study conducted by Baddireddy K and Poojary S showing overall agreement to be 85.0% with κ 0.583 and p<0.001 for CSB when evaluated against KOH for dermatophytosis which was in concordance with the study conducted [8].

In the present study, the CSB 6B stain was found to have better sensitivity, specificity, PPV and NPV of when evaluated against the conventional KOH mount which was in concordance to study conducted by Afshar P et al., [9].

CSB is a rapid method as evident from mean time required for diagnosis with excellent contrast and clear fields as reported by both the independent observers in present study using as simple light microscope. These findings were also reported by previous researchers [1,6,7]. Prakash R et al., have concluded that CSB stain is a better stain for rapid diagnosis of dermatophytes because of ease of performance, rapidity of detection, better appreciation of morphology of fungal elements, and its cost effectiveness [6].

CSB dye has been reported to be corrosive on contact and advisable to use protective gloves while handling the stain but as utilisation of the stain is in low concentrations, as been done in present study, it is unlikely to cause any significant problem [1].

Limitation(s)

This study showed promising results with sufficient samples for superficial skin infections. But, for mycotic keratitis, more number of samples are required to be tested since it throws a challenge for identification of sparse fungal elements from the samples of corneal scrapings.

CONCLUSION(S)

Considering the statistical significance and ease of use of CSB with higher sensitivity with less time required for diagnosis with better colour contrast and clear fields of vison, CSB stain is a better alternative for rapid diagnosis of mycotic infections over the conventional KOH mount. Use of CSB 6B stain being a cost effective with ease and rapidity of diagnosis with better colour contrast helping clinicians with prompt initiation of treatment with a laboratory confirmation should be evaluated with more samples with multi-centric trials and should be regularly used over conventional KOH mount.

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