

Comparison of Two Specimen-Collection and Microscopy Methods for the Rapid Laboratory Diagnosis of Seborrheic Dermatitis- A Pilot Study

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ABSTRACT

BACKGROUND

Malassezia spp. causes seborrheic dermatitis. For laboratory diagnosis, skin scrapings are collected and mounted in potassium hydroxide (KOH) for microscopy and processed for culture. Obtaining scrapings has disadvantages and KOH lacks colour contrast making interpretation difficult. This pilot study compared the results of specimen collection by cellophane tape method with scraping method. It also compared microscopy using Chicago Sky Blue 6B (CSB) stain plus KOH with the conventional method using KOH alone.

METHODS

Skin specimens were collected from the affected sites of 80 patients by scraping and cellophane tape. Specimens were subjected to KOH examination, KOH plus CSB stain, and culture for the presence of *Malassezia spp.*

RESULTS

A total of 160 specimens were collected from 80 patients for microscopy. Of 160 specimens, each was subjected to KOH and CSB plus KOH, 145 (91%) demonstrated *Malassezia spp.* by CSB plus KOH and 124 (77.5%) by KOH alone ($p= 0.001$). Cellophane tape method yielded 141 (88%) positive results compared to 128 (80%) by skin scraping ($p=0.047$). The odds of detecting *Malassezia spp.* was 4.4 times greater when the specimen was collected by cellophane tape and subjected to microscopy with CSB and KOH than when it was collected by scraping and examined microscopically with KOH alone ($p= 0.002$).

CONCLUSIONS

Cellophane tape is a convenient method for specimen collection. CSB stain provides colour contrast and enables easy identification of fungal elements.

KEY WORDS

Cellophane Tape Collection, Chicago Sky Blue 6B Stain, Seborrheic Dermatitis

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BACKGROUND

Malassezia yeasts have been recognized as members of normal human cutaneous flora as well as agents of certain skin diseases. They are lipophilic organisms and have frequently been found on a diverse range of other warm-blooded animals. *Malassezia* genus is classified in the order *Malasseziales* among the *Exobasidiomycetes*. Characteristic features of the genus *Malassezia* include a distinctive morphology and an affinity for lipids in culture. *Malassezia* is implicated in mild but often recurrent cutaneous infections and also have been associated with skin and deep invasive infections in immunocompromised patients. Genus *Malassezia* comprises of 15 species of "yeast like fungi," 13 of which are lipophilic and 1 is non-lipophilic. They are *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. equina*, *M. dermatis*, *M. japonica*, *M. nana*, *M. capre*, *M. yamatoensis*, and most recently *M. cuniculi*, and *Malassezia arunalokei* species.^[1-2]

Seborrheic dermatitis (SD) is the second most common pathology associated with *Malassezia spp.* and its prevalence in the general population is estimated to be 5%. It is a common chronic inflammatory superficial eczematous dermatitis, either sub-acute or chronic, characterized by erythematous plaques with dry or oily scales. This condition is more common in males than in females. Any age is affected especially teenagers and adults. It is seen over areas rich in sebaceous glands-namely, the scalp, face, chest, back and flexural areas. A milder variant is dandruff, which is manifested by dry, flaking scales on the scalp.^[1-2]

The mycological study is usually done by potassium hydroxide (KOH) mount and culture. However, both have their own limitations. Samples for diagnosis are usually collected by direct scraping of skin which is time consuming and sometimes uncomfortable for patients. However, obtaining skin scraping is time-consuming, requires the use of a sharp instrument with its attendant concerns and risk of injury, limits the amount of material collected, and sensitivity and specificity are low. Collection of specimens using a cellophane tape is a useful alternative.^[3-5]

Diagnosis by culture is time-consuming with inconsistent sensitivity.^[6] Direct examination of 10% potassium hydroxide (10% KOH) mount of the specimen is simpler and provides a faster diagnosis but has major drawbacks: it lacks sensitivity, there is interference by artefacts, it requires expertise and is time-consuming.^[6-7] This is chiefly due to the lack of contrast. Chicago Sky Blue 6B (CSB), a direct diazo dye compound, is used as a contrast in various pathology and ophthalmology procedures, and its usefulness has been reported in the diagnosis of pityriasis versicolor. It makes it easy to locate the fungal elements even under scanner view. Another advantage over KOH mount is that it outlines the morphological features of both the spores and the hyphae and can enable detailed study of fungal morphology under oil immersion microscopy.^[8]

This pilot study aimed to compare specimen collection by cellophane tape method and subsequent microscopy by CSB stain and KOH with the routine collection method by scraping and microscopy by KOH alone.

METHODS

This cross-sectional pilot study was carried out at a tertiary care centre over a period of six months. Institutional and ethical approvals were obtained. Given a prevalence of seborrheic dermatitis of 5% in the general population^[2] and taking a confidence level of 95% and power of 80%, a sample size of 80 was derived for this pilot study. Therefore 80 consecutive adult patients (age ≥ 18 years) clinically suspected of having seborrheic dermatitis and presenting to the dermatology clinic during the study period were included. Patients who had been treated with oral or topical antifungal agents in the past one month were excluded.

Specimens were collected by scraping and cellophane tape, one each for KOH mount, KOH and CSB mount, and were cultured on Sabouraud's dextrose agar (SDA) with chloramphenicol and gentamicin overlaying with sterile olive oil. Specimens collected by scraping and cellophane tape were inoculated on tubes and plates of SDA respectively.

Scraping Method

After cleaning the affected area with 70% isopropyl alcohol, skin scrapings were taken from the edge of the lesion with the blunt edge of a sterile scalpel blade (no. 10). Scrapings were placed on two clean microscopy slides, one of which had three drops of 10% KOH solution, and the other 10% KOH with CSB stain (Sigma-Aldrich, USA). CSB stain was prepared by dissolving 1 gm CSB 6B powder (Sigma-Aldrich, USA) in 100 ml distilled water. For culture, specimens were collected on a sterile Whatman filter paper.

Cellophane Tape Method

Two 5 cm x 2 cm strips of clear transparent cellophane tape (Wonder Tape 555, Wonder Tape Industries, India) were applied over the affected site, pressed firmly, removed. Then they were then stuck on the surface of two clean microscopy slides, one with 10% KOH solution, and the other with 10% KOH plus CSB stain. Specimens for culture were taken from the skin by stripping with a third 1 cm x 1 cm strip of tape.

Microscopy

The slides were visualized under low power (10x magnification) as well as high power (40x magnification). The presence of hyphae and spores exhibiting characteristic "spaghetti and meatballs" appearance was considered positive for the presence of *Malassezia spp.*^[9]

Culture

Specimens collected by scraping and cellophane tape were inoculated on tubes and plates respectively of Sabouraud's dextrose agar (SDA) with chloramphenicol and gentamicin (HiMedia; Mumbai, India). They were overlaid with sterile olive oil and they were incubated at 32°C for seven days.^[9-10] Growth of *Malassezia spp.* was confirmed by colony morphology, urease, catalase tests and safranin staining.^[9,10]

Statistical Analysis

Data was analysed using SPSS (v16.0; SPSS Inc; Chicago, USA). Descriptive statistics, median and percentages, were calculated to describe demographic data. The categorical

variables were compared using Pearson's χ^2 test or McNemar test when appropriate. p value ≤ 0.05 was considered significant.

Time to detection in positive specimens was calculated from the moment the specimen was collected by cellophane tape or scalpel to the moment characteristic morphology was observed.

RESULTS

Of the 80 patients who were included, 46 (58%) were in the age group 18-30 years. Median age was 29 years. Forty-six patients (58%) were males and 34 were females. Male to female ratio was 1.35:1 with a slight male preponderance. Five seborrheic dermatitis patients reported exposure to sunlight as a predisposing factor for seborrheic dermatitis. No other history of allergies to oil, talcum powder, shampoos or predisposing factors like temperature or heat difference could be elicited. The most common site affected in SD patients was scalp, followed by neck, back, forehead and nose. All patients (100%) had scaly lesions, 64 (80%) had itchy lesions and 67 (84%) had hypo-pigmented lesions. The commonly affected sites were scalp (60%), neck (45%), back (33%), forehead (28%) and nose (25%).

Malassezia spp. was detected by microscopy in 73 (91%) patients, and by culture in 35 (44%) patients ($p < 0.001$). The difference between microscopy and culture for detecting *Malassezia*, was statistically significant. Of 160 specimens collected by each method, *Malassezia spp.* was detected in 141 (88%) collected by cellophane tape and in 128 (80%) collected by scraping ($p = 0.047$). *Malassezia spp.* was detected in 145 (91%) of 160 specimens mounted in KOH with CSB, compared to 124 (78%) of 160 mounted in KOH alone ($p = 0.001$). The odds of detecting *Malassezia spp.* was 4.4 times greater when the specimen was collected by cellophane tape and subjected to microscopy with CSB and KOH than when it was collected by scraping and examined microscopically with KOH alone ($p = 0.002$) (table 1). The median time to detection in positive specimens for collection by cellophane tape method and microscopic examination CSB and KOH was two minutes, whilst that for collection by scraping and examination by KOH was five minutes.

Method	Positive	Negative	Total
Cellophane tape & CSB	73 (91%)	7 (9%)	80
Scalpel & KOH	56 (70%)	24 (30%)	80

Table 1. Comparison of the Two Collection and Staining Methods

DISCUSSION

Seborrheic dermatitis (SD) is a common, relapsing dermatitis affecting sebum-rich areas of the skin and is caused by fungi of the *Malassezia spp.* It is a common, relapsing dermatitis that is characterized by erythematous patches and superficial scaling. It affects sebaceous-rich gland areas – namely the scalp, face, central chest and anogenital areas. Adult seborrheic dermatitis is reported to involve one or more sites which includes scalp, eyebrows, forehead, nasal alar creases, chest and genital region. [9]

Seborrheic dermatitis is a chronic, recurrent condition, and patients generally experience frequent relapses. Usually seborrheic dermatitis is mild and responsive to therapy. But in severe or atypical cases, a variety of other entities should be considered in the differential diagnosis. The clinical presentation, though very typical, may be confused with other skin disorders like psoriasis, atopic dermatitis, contact dermatitis, and erythrasma and treatment for each differs. The diagnosis of seborrheic dermatitis is based on typical clinical picture, positive direct microscopy and culture. Isolation of *Malassezia spp.* from clinical specimens remains a challenge because of their lipid dependency, making culture a time-consuming exercise with inconsistent results and a low sensitivity. The cultures are considered positive for *Malassezia* if there is growth of cream to yellowish brown colonies, confirmed by morphological and biochemical tests. False negative findings in culture may arise when specimen contains dead or nonviable mycotic organisms or when the specimen is not enough. Therefore, as compared to culture, direct microscopic examination of specimens could be considered as the simplest and most inexpensive method for the laboratory diagnosis of *Malassezia*. [6-7]

Seborrheic dermatitis should always be confirmed with direct microscopy or culture to demonstrate pseudohyphae and blastoconidia in typical “spaghetti and meatballs” pattern. Direct microscopic examination of specimens is the simplest and most inexpensive method for the laboratory diagnosis of *Malassezia spp.* Direct examination of skin scraping by KOH examination is done for demonstration of fungal spores and hyphae. But this needs expertise as there is no contrast. [6-7] Skin scrapings from the affected area are the usual specimens submitted for microscopy. However, this method has disadvantages which affect the sensitivity and specificity of microscopy: only a relatively small area of the skin can be sampled, quantity of specimen collected is less, and microscopy of skin scrapings is compounded by the presence of artefacts (especially due to crystallisation of KOH or “ferning”). Additionally, the method demands manual dexterity, is time-consuming and there is risk of injury to the patient and patient concern arising from the use of a sharp instrument like a scalpel blade. [11-12]

Cellophane tape method was first used for the diagnosis of tinea versicolor and later its superiority was demonstrated for the diagnosis of dermatophytosis and superficial candidiasis. [4,13-15] Miranda et al. [12] reported a diagnostic sensitivity of 98.3% for superficial mycoses. In the present study, the diagnostic yield of *Malassezia spp.* from specimens collected by cellophane tape was significantly greater than that from scrapings. Cello tape method is also a relatively safer option for specimen collection and is suitable for the collection of sufficient quantity of skin specimen and is particularly useful if the examiner is inexperienced. Besides being a safer method than scraping, cellophane tape collection enables sampling of a larger area, preserves fungal morphology better and prevents clumping, thereby increasing sensitivity. In addition, it has excellent sensitivity & accuracy of examination and the fungal hyphae are minimally distorted by this technique. [3,11] Also, this is a simpler method requiring no special dexterity or training, takes less time and facilitates transportation and storage. [13,16] It is also effective for direct microscopic diagnosis of chromomycosis, lobomycosis, and para-

coccidioidomycosis, Pityriasis versicolor, dermatophytosis, *Enterobius vermicularis* etc. But it has few disadvantages like specimen collection from moist highly inflamed areas is difficult and excessive handling of the sticky surface of the tape or the presence of oil or grease may lead to opacification of the tape.^[16]

Various contrast agents have been evaluated for the diagnosis of superficial mycoses. Chlorazol-KOH testing has low sensitivity and specificity and may be carcinogenic, whilst acridine orange and calcofluor white require fluorescent microscopy.^[15,17] Chicago Sky Blue 6B (CSB) is a diazo dye generally used as a counterstain in immunofluorescence histochemistry, to assess viability of spermatozoa in animal studies and for dye-assisted macular surgery. CSB stain was used by Lodha et al, for rapid diagnosis of pityriasis versicolor by providing a colour contrast.^[8] Several workers have reported increased sensitivity and specificity of microscopy using CSB in the diagnosis of dermatophytosis and superficial candidiasis.^[8,15,17,18] In the present study, we found that addition of CSB to KOH provided a colour contrast that made reading and interpretation of slides, easier and took lesser time to interpret. It highlighted morphological features of fungi, superiorly. It was easy to perform and required only an ordinary light microscope and hence can be a good a cost effective, clinic-based test for rapid diagnosis of seborrheic dermatitis. CSB stain significantly increased the diagnostic yield for seborrheic dermatitis over a plain KOH mount.

Our study has a few drawbacks. This was a pilot study with the methods being assessed on the same lesions from the same patients with no randomisation or blinding. A larger randomised controlled study with blinding as well as a cost assessment would be more conclusive.

CONCLUSIONS

Specimen collection by cellophane tape increased the diagnostic yield of *Malassezia spp.* and was easier than scrapings. Microscopy with CSB stain significantly increased the diagnostic yield for seborrheic dermatitis over microscopy with plain KOH mount.

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