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Original Research Article

Cellophane Tape Pugh's Staining a Novel Contrast Staining Method for Rapid Detection of Fungal Elements of *Malassezia* in Pityriasis Versicolor

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ABSTRACT

Background: Pityriasis versicolor (PV) is a chronic superficial fungal disease of the stratum corneum etiologically associated with the *Malassezia* spp. As the routinely used KOH wet mount is time consuming and requires an expert to interpret the results, a new staining method that can replace KOH wet mount has to be found out.
Objective: To demonstrate the diagnostic efficacy of cellophane tape Pugh's staining in comparison with routinely used KOH wet mount for the diagnosis of PV.
Methods: A total number of 450 specimens from different body sites of 422 PV cases with respect to colour of the lesions were studied. Skin scrapings were subjected to KOH wet mount and cellophane tape stripping and staining method was used in case of Pugh's staining. Statistical analysis was done.
Result: Both the KOH wet mount and cellophane tape Pugh's staining gave similar results. Out of 450 lesions sampled, 402 (89.3%) gave positive results. Fungal elements were seen more clearly in purple colour of the stain. The sensitivity and specificity of Pugh's stain when compared with KOH wet mount was 100%.
Conclusion: Cellophane tape Pugh's staining used as a novel method in this study has the potential to replace the KOH wet mount, as the routine method for the rapid diagnosis of PV.

Key Words:- Pityriasis versicolor, *Malassezia,* KOH wet mount, Pugh's stain

INTRODUCTION

Pityriasis versicolor (PV) is one of the most common superficial fungal infection prevalent in tropical and subtropical countries etiologically connected to lipophilic fungi of the genus *Malassezia*. [1-3] The disease manifest clinically as asymptomatic hypo/ hyperpigmented macules or a combination of the two that are covered by fine scales (*pityron*, Greek for scale) usually affecting seborrheic areas of the skin surface, such as the back, chest, and neck. [1,4] PV is easy to diagnose but should always be confirmed by direct examination with10% potassium hydroxide (KOH) wet mount. Addition of an equal amount of Parker blue/black Quink permanent fountain pen ink to KOH (Parkers stain) enhances the visibility of the same. [2] Similarly, methylene blue stain could be added to the KOH preparation. [2,5] When observed with 10% KOH mixed with a fluorescent brightener, Calcoflour white fluorescence is observed under a fluorescent microscope. [3,6] Another method to stain fungal elements in PV which has proved to

be as effective as KOH mount has been described by Payale B *et al.* in 1994. This method involves the application of Alberts solution "A" containing toluidine blue and malachite green on the material taken by the scotch tape stripping technique. [7] A new contrast stain containing 1% Chicago sky blue 6B and 8% KOH as the clearing agent was used in 2008 by Lim SL and Lim CS in Australia. [8] Wiliam Thomas Gordon Pugh in 1905 had described simple staining technique, using toluidine blue in absolute alcohol and glacial acetic acid for the detection of the diphtheria bacillus by demonstration of its volutin granules. [9] This study reports the use of Pugh's stain (which was originally used for the identification of volutin granules of diphtheria bacilli) for the demonstration of fungal elements of *Malassezia* in PV. To prove its efficacy, it was compared with routinely used KOH wet mount. To the best of our knowledge, this is the first study comparing the utility of cellophane tape Pugh's staining with KOH wet mount for the rapid detection of fungal elements of *Malassezia* in PV.

MATERIALS AND METHODS

The study was conducted at a tertiary care hospital in North Kerala. The study group comprised of 422 clinically suspected pityriasis versicolor patients attending Skin and Venereal outpatient department during one year period. A total number of 450 specimens from different body sites of 422 PV cases with respect to colour of the lesions were studied.

Two types of direct microscopic preparations were undertaken: (i) using 10% potassium hydroxide (KOH) (ii) using Cellophane tape Pugh's staining. The entire preparations were microscopically examined by experienced personnel and were considered positive if the fungal elements (yeast cells and short thick hyphae) could be identified. KOH wet mount [10] was done by scraping thePV lesions with sterile No: 15 scalpel blades without disrupting healthy looking areas. The scrapings were placed on a glass slide with1–2 drops of 10% KOH. A cover slip was placed over the mixture and the slide was examined under the 40X objective of the microscope for fungal elements after a few minutes. Cellophane tape Pugh's staining was performed by lightly touching with a piece of clear transparent cellophane tape, sticky side down and pressing on the lesions. A drop of Pugh's stain (10% toluidine blue in absolute alcohol and 5% glacial acetic acid) [9] was placed on a clean glass slide. The adhesive side of a clear cellophane tape was placed on to the lesion and then applied gently onto a drop of stain on a clean glass slide. The preparation was observed under the 100X objective of the microscope and the presences of for fungal elements were documented. Results obtained were tabulated and statistical evaluation of the results were done.

RESULTS

This study compared two different microscopic methods i.e.; KOH wet mount and cellophane tape Pugh's stain, for direct microscopic study of PV. Since KOH wet mount is the commonly used existing method for identifying PV. Therefore KOH wet mount has been considered as the gold standard and Pugh's stain method is compared to this. Both the KOH wet mount and cellophane tape Pugh's staining gave similar results. Out of 450 lesions sampled, 402 (89.3%) gave positive results and 48(10.7%) were negative. (Figure – 1), (Plate: 1, 2).

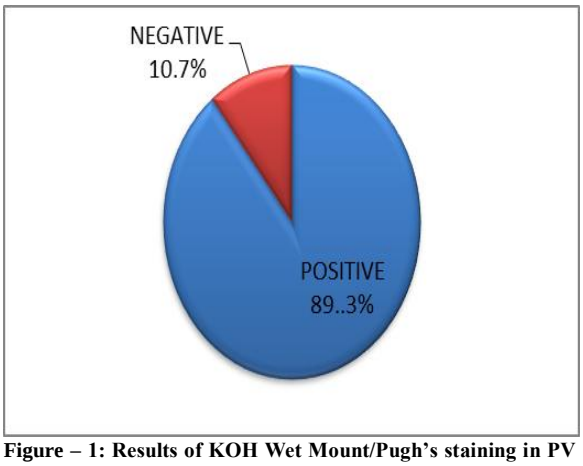

Figure – 1: Results of KOH Wet Mount/Pugh's staining in PV Cases

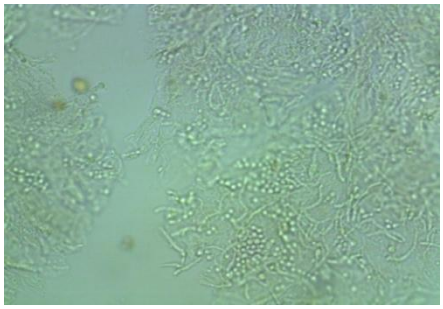

Plate: 1.Direct microscopy using KOH wetmount showing hyphae and yeast cells of *Malassezia* in PV

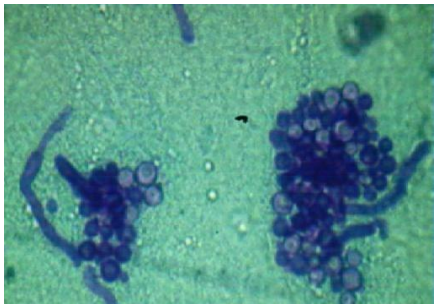

Plate: 2.Direct microscopy using cello – phane tape Pugh's staining showing pseudo-hyphae and yeast cells of *Malassezia* in PV

All 402 cases which were found positive using KOH were also found to be positive using Pugh's staining. Similarly all 48 cases which were found negative using KOH were also found to be negative using Pugh's staining (Table –I). The sensitivity and specificity of Pugh's stain when compared with KOH wet mount was 100%.

Table- I: Diagnostic efficacy of Pugh's stain Compared to KOH Wet Mount

Pityriasis Versicolor Cases		KOH wet mount		Total
		Positive	Negative	
Pugh's Staining	Positive	402	0	402
	Negative	0	48	48
Total		402	48	450

Although results of cellophane tape Pugh's stain were similar to KOH wet mount, the fungal elements were seen more clearly in purple colour of the stain and consequently the technique was found to be more effective. Furthermore, the yeast cell morphology (round / oval shape) can be clearly elucidated. Yeast cells are seen larger, when observed under oil immersion objective (100X) than KOH wet mount which is observed under (40X) objective.

DISCUSSION

The differential diagnosis of pityriasis versicolor includes skin infections like vitiligo, idiopathic guttate hypomelanosis, cholasma (melasma) on the face, hypo-or hyperpigmented mycoses fungoides, pityriasis alba, pityriasis rotunda or pityriasisrosea, tinea corporis and secondary syphilis. [11] The disease can be easily diagnosed by an experienced dermatologist, but this should be always be confirmed with direct microscopy to demonstrate pseudohyphae and blastoconidia in typical "spaghetti and meatballs" pattern. [2,11] Contrast stains, including Parker blue black ink, [2,11] methylene blue, [5] calcofluor white, [3,11] Chicago sky blue 6B (CSB) mixed with KOH [8] have been used in the diagnosis of PV. Currently available of Parker ink both blue and black in Kerala, India do not seem to work well as it produces a red solution when mixed with KOH. This may be due to some change in manufacturer's formula. Calcofluor white, is expensive as it requires a fluorescent microscope. [2,11] Another method to stain fungal elements in PV cases is Albert's solution "A" containing toluidine blue and malachite green which has proved to be as effective as KOH wet mount. [7] This study make use of Pugh's stain which was first described by Wiliam Thomas Gordon Pugh in 1905 and was originally used to study the metachromatic granules of *C. diptheriae*. [9] This is a simple staining technique, using toluidine blue in absolute alcohol and glacial acetic acid, without malachite green which is present in Albert solution A. This study compared cellophane tape Pugh's stain with KOH wet mount in patients with clinical diagnosis of PV.

The present study showed KOH positivity in majority (89.33%) of the PV lesions studied and this is at par with the Indian studies from Punjab [12] and Varanasi. [13] An Indian study from Karnataka, [14] Iran [15] and Egypt. [16] demonstrated presence of yeast cells with short hyphae in all the PV cases studied (100%) by direct microscopy. A study from Bosnia and Herzegovina, [17]

and two Iranian studies [5,18] showed the presence of yeast cells and short filaments in direct microscopy 97.8%, 98.9%, 94.9% of the samples respectively. On the other hand, lower rate of detection than this study have also been obtained in other studies. [19,20]

The sensitivity and specificity of Pugh's stain when compared with KOH wet mount is100%. No studies are available regarding the comparison of these methods. The new method is a staining method that can colour the blastoconidia and fungal elements, and so the fungal cell morphology can be visualized better /clearly elucidated and differentiated from background keratinocytes. KOH wet mount does not produce a colour contrast and requires an expert to interpret and requires more time than cellophane tape Pugh's staining.

This method is simple (easy to perform), rapid, less time consuming as it can be completed within one minute and thus allows for a faster reading than KOH wet mount which takes 4 – 5 minutes for screening each slide. It is sensitive, cost effective (not requiring the use of scalpel), require only a piece of (4 cm) cello tape, a drop of stain and a glass slide and requires only an ordinary light microscope and 10 mL of Pugh's stain is enough for 200 smears and hence the method is inexpensive). It is also a highly reliable method, for identifying fungal elements of *Malassezia* from PV. As this is observed under oil immersion (100X) objective; fungal elements (Yeast cells and short thick hyphae) are seen larger can be easily differentiated from background cell debris materials. This study reports cellophane tape Pugh's staining to be as effective as 10% KOH wet mount and recommends this as a method for the diagnosis of PV with the above added advantages. Additionally, the specimen collection is easier in case of cellophane tape Pugh's staining as it uses pressing with cellophane tape instead of scraping with scalpel blades. So this method is very useful in case of facial lesions particularly in children. This staining method has the potential to replace the KOH wet mount, as the routine method for the rapid diagnosis of PV.

CONCLUSION

Cellophane tape Pugh's staining used as a novel method in this study has the potential to replace the KOH wet mount, as the routine method for the rapid diagnosis of PV. This method is very simple, economical, rapid, non-laborious, sensitive and cost effective. Being a staining method that can colour blastoconidia and fungal hyphae, the fungal cell morphology can be clearly elucidated and differentiated from background keratinocytes. So we recommend this technique for the routine diagnosis of pityriasis versicolor.

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