Evaluating diagnostic utility of PAS stained skin scrape cytology smear in clinically suspected superficial cutaneous mycoses: A simple yet unpracticed technique

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Abstract

Background and Objectives: For fungal dermatitis, a wet Potassium Hydroxide (KOH) preparation of the skin scrapings forms the routine practice for diagnosis. This study was carried out to determine the diagnostic accuracy and to evaluate the value-added information of PAS-stained scrape cytology smear in evaluating the presence of fungal elements. Methods: This prospective study was carried out on the patients clinically diagnosed with superficial cutaneous mycoses. For each patient, a wet KOH preparation and one PAS-stained skin scrape cytology (SSC) smear was prepared. Results: Out of the 52 suspected cases of superficial cutaneous mycoses, 50 showed fungal elements on either or both the techniques. The presentation was Tinea cruris together with Tinea corporis in 21 cases (42%), isolated tinea cruris in 19 cases (38%), tinea corporis in 8 cases (16%), and tinea corporis with tinea manuum and onychomycosis in 2 cases (4%). KOH preparation was positive in 45 out of 50 cases (90%) and SSC was positive in 49 out of 50 cases (98%). The sensitivity, specificity, positive predictive value, and negative predictive value for PAS-stained SSC smear was 98%, 100%, 100%, and 66% and that for KOH preparation was 90%, 100%, 100%, and 28%, respectively. Fungal elements quantity was graded on SSC smear as 1+(5), 2+(19), 3+(19), 4+(6), and in one case, it was negative. Conclusion: Incorporation of the PAS-stained SSC smear for fungal dermatitis ensures more efficient and confident diagnosis and the slides are available for archivable studies.

Keywords: Cytology, dermatophytosis, KOH preparation, PAS stain, scrape cytology

Introduction

Fungal skin infections form a significant burden of the routine dermatology cases encountered by the primary care physicians, and these have been broadly classified into two types: superficial and deep type.¹,² Superficial mycoses are further classified into two types: surface and cutaneous. Similarly, deep mycoses are of two types: subcutaneous and systemic. The three common dermatophyte species that cause superficial cutaneous mycoses are Microsporum, Trichophyton, and Epidermophyton. Candida spp., can lead to superficial as well as deep mycoses. The common fungal types causing subcutaneous deep mycoses are Aspergillus, Zygomycosis, Rhinosporidium spp., and chromomycoses. Systemic infections with Cryptococcus and Histoplasma may involve skin secondarily.
Cytologic methods can be utilized in the diagnosis of various dermatologic diseases.[1] Tzanck is a modified scrape smear that is widely utilized for vesiculobullous disorders and even for some solid malignancies.[4–8] Slit skin smear with incorporation of lepra stain has been widely used in clinically suspected leprosy patients. Fine needle aspiration cytology has been utilized for any palpable swelling of the skin. Skin scrape cytology (SSC) is the method of choice for superficial lesions involving epidermis.

For superficial cutaneous mycoses, a skin scrape and a KOH preparation form the routine practice for diagnosis.[9] The biopsy which is usually contraindicated as fungal dermatitis involves only stratum corneum and scar is sequelae of skin biopsy. SSC together with fungal stain can not only provide a diagnosis but also helps in looking at the skin cellular elements and inflammatory components.[7–9] Periodic Schiff’s (PAS) reagent is a special stain that has been found to be very helpful in histopathology of skin biopsies; however, its role in cutaneous cytology has not been much described in the literature.[10] PAS is a carbohydrate stain that reacts with polysaccharides and chitin in the capsules or walls of many fungal species.[11] The fungal elements are PAS positive and diastase resistant.

The laboratory diagnosis of fungal infections encompasses direct microscopic examination, fungal culture techniques, and non culture methods.[11,12] Direct microscopic examination includes examination of wet mounts—KOH, Chicago sky blue stain, Parker blue-black ink, Swartz-Lamkins stain, calcofluor white, India ink—fluorescent antibody staining, and histopathology. Non culture methods include serology tests for cell-mediated immunity and molecular methods.

The present study aims at evaluating the utility of PAS-stained SSC smear in clinically suspected fungal infections of the skin. Also, the study aimed to evaluate the value-added information of SSC in comparison to KOH preparation.

Material and Methods

After taking due approval from research and review board and institute ethics committee, the study was carried out in a span of 3 months on the patients presenting to the department of Dermatology of a hospital in Central India. The patients included were those who were clinically diagnosed with fungal dermatitis. Many of the cases had received some or the other kind of treatment. After taking an informed consent in the patients’ own vernacular language, the patient’s clinical details and demographic profile was noted in the case study form.

Inclusion criteria

All cases of clinically suspected superficial fungal infections of skin are included in this study.

Exclusion criteria

Fungal infection of the nails and scalp were excluded as crushing and fixation of nails and hair onto slides would not have been possible. Also, the cases in which both the techniques could not be carried out were excluded.

Technique for obtaining scrape

The scrape was obtained by the dermatologist under all aseptic precautions. After thorough cleaning of the lesional area with alcohol swab, scrapings were taken from the active edges of lesion with the help of a scraper or edge of a slide. The gentle scales were preferred for cytology smear.

Technique for KOH preparation (Direct microscopic examination)

The scrapings were put in the center of the slide and a square shaped cover slip was placed over it. A drop or two of 10% KOH was put from the edge of the cover slide. Gentle warming of the slide was done with the help of spirit lamp. It was then kept at room temperature for 5 to 10 min. The slide was viewed first at low power field with the condenser low and illumination reduced. Then, it was seen at high power field. The presence of branching hyaline septate hyphae was considered as a positive test for fungi.

Skin scrape cytology

The scrapings were crushed with the help of another slide (technique used same as for spreading aspirated material) so as to ensure they stick to slides. Steps used for doing PAS stain are as follows:

1. The slides were put on glass rods at a distance of at least 1 inch from one another. Spray fixative/alcohol fixative is put on the slide for at least half an hour to an hour. The slides usually dried on their own.
2. After that 0.5% periodic acid solution was put on the slides for 5 min at room temperature.
3. Gently rinse the slides by pouring distilled water over it.
4. Put Schiff’s reagent on the smear to fully cover the slide for 15 min at room temperature
5. Rinse slides in tap water
6. Counterstain slides in hematoxylin slides for 2 min (in a coplin jar)
7. Rinse slides in running tap water
8. Dehydrate, clear, and mount

Reporting of PAS-stained SSC

Pink hyphal structures and spores attached to the squamous cells were considered as positive. Numerous yeast forms, if present attached to epidermal cells, were also considered positive. A note was taken of the presence of inflammatory cells and bacterial colonization. Grading of fungal elements was done as follows:

• 1+=Occasional but definite fungal hyphae attached to epidermal cells.
• 2+=Easily identifiable hyphae at low power.
• 3+=Numerous hyphae crossing the epidermal plates seen on scanner.
• 4+=The hyphae are so numerous that they are closely packed, touching, and overriding each other.
Results

This study was conducted in Bhopal, Central India, in the months of May to July. Out of the 52 suspected cases of fungal infections of skin, 50 showed fungal elements on either or both the techniques. In the rest of the two cases in which fungal elements were not seen, an additional punch biopsy was taken. The histopathological examination along with fungal stains also confirmed the absence of superficial mycoses. Both the cases were diagnosed as allergic contact dermatitis on histopathological examination. The clinical expertise was correct in 96% cases.

Age and gender distribution

These 50 patients aged in range from 1 to 62 years with a mean age of 28.8 years. Almost 66% (33 cases) of the cases were falling in the 11 to 30 years range and 28% (14 cases) were falling in the 31 to 50 years range. Male to female ratio was 2.57:1. There were 2 cases of pityriasis versicolor and both were seen in young girls. Most of the patients belonged to the middle class (26; 52%), followed by those from lower class (20; 40%) and upper class (4; 8%). Most of the patients were students (18 = 36%), followed by farmers (6 = 12%), housewives (6 = 12%) etc., and only one was a minor child. The duration of lesions ranged from few days to the maximum of 8 years. Thirty-seven (74%) of the patients gave history of duration of lesions in months, 8 (16%) gave duration of lesions in years, and 5 (10%) gave history of duration of lesions to be in days.

The clinical presentation was Tinea cruris together with Tinea corporis in 20 cases (40%), isolated T. cruris in 18 cases (36%), T. corporis in 7 cases (14%), T. cruris with T. corporis with T. facei in 1 case (2%), T. facei in 1 case (2%), tinea corporis with tinea manuum with onychomycosis in 1 case (2%), and tinea manuum with onychomycosis in 1 case (2%). Multiple regions of involvement were seen in 28 cases (56%). Because of the multiple regions involved, the clinical patterns involved were more than the number of patients. Total number of clinical patterns seen were 76: T. cruris 40 (52%), T. corporis 29 (38%), T. facei 3 (3.9%), T. manuum 2 (2.6%), and Onychomycosis 2 (2.6%). There were 2 cases of pityriasis versicolor and both were seen in upper part of body [Figure 1].

The lesions were erythematous annular papular, patch, plaque type in 42 (84%) cases, hyperpigmented with blackening in 5 (10%) cases, and hypopigmented macular in 3 (6%) cases. These three cases in which hypopigmentation was seen, two of them were cases of pityriasis versicolor, and in one case yeast forms were more than hyphal forms.

Out of the 50 patients, 13 (26%) had not received any kind of treatment for skin infection. Rest were using on and off topical antifungal medications. Comorbid conditions were seen in six (12%) patients out of which diabetes was the most common (three of them were diabetic, one was post varicella (isoloci response), one of the patients was on oral steroids, and one baby girl was with protein energy malnutrition) [Figure 2]. Forty four (88%) patients had no obvious comorbidities.

KOH preparation was positive in 45 out of 50 cases (90%). The sensitivity, specificity, positive predictive value, and negative predictive value for KOH preparation was 90%, 100%, 100%, and 28%, respectively.

PAS-stained SSC was positive in 49 out of 50 cases (98%). The sensitivity, specificity, positive predictive value, and negative predictive value for this technique was 98%, 100%, 100%, and 66%. Fungal elements quantity was graded on SSC smear as 1+(5), 2+(19), 3+(19), and 4+(6) and in one case, it was negative [Figure 3]. The five cases in which KOH was found to be negative were having low grade positivity (two cases having 2+ positivity and 3 cases having 1 + positivity). Out of the total 49 cases that were positive on PAS, 45 cases showed hyphae only or hyphae with conidia formation [Figure 4] and two cases showed “spaghetti and meat balls” appearance. Inflammatory components were seen in 5 (10%) out of the 50 patients [Figure 2d] and 2 (4%) cases showed additional bacterial colonies. Fungal hyphae in the hair shafts sampled during scrapings were seen in 2 (4%) cases.

More fungal hyphae were highlighted and fungal hyphae were more easily recognized on low power magnification in comparison to KOH [Figure 5 and Table 1]. The average turnaround time taken for KOH preparation was 20 min and that of PAS-stained SSC smear preparation was 1 h 40 min. The approximate amount of money spent for PAS stain for each case was Rs. 90 to 130 (INR) and for KOH preparation will be Rs. 14 to 16 (INR). Although the technical labor costs were not added.

Species identification was not the aim of the study and culture characteristics were not available in many of the cases.
Higher incidence in males has been reported in several studies; this could be due to greater physical activity and increased sweating. Mostly patients belonged to middle class followed by lower class and then by upper class, and middle class preponderance has been noted in an earlier study. Six patients had associated comorbidities and other studies has reported the associated diseases like diabetes mellitus, atopic diathesis, and HIV infection. Tinea has also been reported in site of healed herpes zoster. Scrape cytology of nasal polypi and skin ulcer together with PAS stain has been found to be particularly useful in rapid and correct diagnosis of rhinosporidiosis. A correct preoperative diagnosis facilitates the planning of surgical interventions to prevent recurrences. The positivity by KOH reported varies from 59.66% to 96%, and in our study it was 90% [15–17,20,21] [Table 2]. And the sensitivity of SSC was still higher. We excluded samples from nail and hair.

Discussion

This study was conducted in Bhopal, India, in the months of May to July. The temperature in Bhopal in these 3 months varied from coldest 23.2°C to warmest 40.6°C and the relative humidity percentage varied from 20% to 90%. The hot and humid climate is more favorable for the development of superficial mycoses. In this study of 50 cases of dermatophytic infections, the following clinical forms were observed: Tinea corporis, tinea cruris, tinea manuum, tinea faciei, and onychomycosis. As regard to the age incidence, maximum number of cases encountered was in the age group of 11 to 30 years (66%). This is in accordance with the findings of other workers. This was followed by the age group of 31 to 50 years (28%). Overall, a male predominance was noted.

Two cases, characteristic “spaghetti and meat balls” / “bananas and bunch of grapes” appearance lead to the Malassezia spp. identification. PAS-stained SSC is a good technique to identify yeasts and pseudohyphae of Candida spp. but these were not seen in this study. All the cases seen had slender septate hyphae with branching. Formation of arthropores were seen.
as these will not crush and fixation to the slide will be difficult, that is, why it was our exclusion criteria. We only took samples of fungal dermatitis. Although an attempt at KOH-treated nail clippings followed by PAS stain can be done as has been described in earlier studies. The culture positivity varies from 39% to 62.7% as described in literature. Culture is recommended even if KOH is negative because it may lead to diagnosis in those cases where KOH is negative and species identification is possible with culture methods. *Trichophyton rubrum* has been found to be the most common species in Indian set up followed by *Trichophyton mentagrophytes*.

Some precautions that needs to be followed during PAS staining on SSC are as follows: The scrapings should be crushed to ensure that at least some scrapings will stick onto slides. The slides should be kept on glass rods at a distance of 1 inch because if the slides are closely placed, scrapings of one slide may float to the other while fixing. If the slides are put in coplin jar for fixation, the scrapings might float away and may give a false negative diagnosis. A prolonged fixation of at least 30 min to 45 min ensures best results. All the staining should be carried out on glass rods, only the staining in haematoxylin and thereafter dehydration can be carried out in the coplin jars.

While interpreting, it should be kept in mind that starch granules of glove powder are also PAS positive and have a diamond-shaped refractile center and they should not be mistaken for yeast forms. The cytoplasm of epidermal cells because of the glycogen content is also PAS positive which serves as internal control for the quality of PAS stain.

Although SSC/Tzanck is an old technique, PAS stain has been widely utilized in histopathology in suspected fungal infections. The combined technique of PAS-stained SSC has not been evaluated in diagnosing clinically suspected fungal infections of skin. This work is about adapting an existing technique to the realm of skin disease and not inventing a new technique entirely. This technique can be used for suspected superficial mycoses which is a common disease in the setting of primary care.

The limitation of this study was that there were far less number of negative controls.

To the best of our understanding, this is the first study utilizing the novel concept of utilizing the PAS stain on SSC in clinically suspected fungal infections of skin. The study concludes that PAS-stained SSC is a more sensitive method than conventional KOH preparation. The results are not subjective and give a more confident diagnosis. Its use should be encouraged as it gives permanent slides within 2 h which are a permanent record and can be archived at a later stage. The authors recommend the practice of this test technique in suspected superficial mycoses.

Table 1: Comparison of PAS-stained skin scrape cytology smear and KOH technique

| Variable                                      | PAS-stained SSC | KOH                      |
|-----------------------------------------------|-----------------|--------------------------|
| Type of slides                                | Permanent       | Wet preparation          |
| Grading of fungal elements quantity           | Possible        | Not possible              |
| Species identification                        | Not possible    | Not possible              |
| Sensitivity                                   | 98%             | 90%                      |
| TAT (Turnaround time)                         | 1 h 40 min      | 20 min                   |
| Additional findings                           | Inflammatory cells can be seen | Not seen |
| False impression of fungal hyphae             | Not there       | Likely possible (Mosaic fungus) |
| Expertise for staining/preparation required   | Required        | Not required              |
| Staining of hard tissue like nails            | Crushing not possible, so staining not possible on cytology. | Good for nails, hair, and harder tissues |

Table 2: Comparison of the indexed study with the previously published studies from India

| City                | A Naglot et al.[15] | Singh S et al.[16] | Bindu et al.[17] | Surendran et al.[18] | Peerapur et al.[19] | Present study |
|---------------------|----------------------|--------------------|------------------|----------------------|---------------------|---------------|
| No. of patients     | 632                  | 260                | 150              | 100                  | 102                 | 50            |
| Sex ratio (M:F)     | 2.2:1                | 1.57:1             | 2.06:1           | 1.63:1               | 2.5:1               | 2.5:1         |
| Age distribution    | Not given            | Not given          | 13 day-75 years  | 6-60 years           | Not given           | 1-62 years    |
| Most common decade  | 21-30 years, 31-40 years (% not given) | 16-30 years (45.38%) | 11-20 years (23.3%) | 16-30 years (44%) | Not given           | 21-30 years (38%) |
| of presentation     | T. corporis (34.82%) | T. corporis most common (% age not given) | T. corporis (54.6%) | T. corporis (44.3%) | T. corporis with T. cruris with T. corporis (40%) | T. cruris with T. corporis (10%) |
| KOH positivity      | 59.66% (KOH and culture) | 60.38%             | 64% positivity   | 96%                  | 74.5%, 40%          | Not done      |
| Culture positivity  | 59.66% (KOH and culture) | 44.62%             | 45.3%            | 39%                  | 62.7%              | Not done      |
| Species identified  | T. rubrum (50.15%)  | T. rubrum           | T. rubrum (66.2%) | T. rubrum (67.5%)    | T. rubrum (43.7%)   | Not done      |
| PAS stain sensitivity| Not done            | Not done            | Not done         | Not done             | Not done           | 98%           |

The authors: Khurana et al.: PAS-stained skin scrape cytology smear

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1093
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Declaration of patient consent
An informed written consent was obtained from our patients for publishing the clinical photographs.

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Conflicts of interest
There are no conflicts of interest.

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