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Original Research Article

Utility of Fine-needle aspiration cytology in diagnosis of lymphocutaneous sporotrichsis

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Abstract

Objective: Isolated case reports describing the utility of Fine Needle Aspiration Cytology (FNAC) in the diagnosis of Lymphocutaneous sporotrichosis are available in the literature. Since Himachal Pradesh in India is a known endemic area for cutaneous sporotrichosis, this study was conducted to assess the utility of FNAC in the detection of fungal spores for the diagnosis of lymphocutaneous sporotrichosis.

Methods: FNAC and biopsy were performed from the nodules and indurated ulcers of fifty clinically diagnosed cases of lymphocutaneous sporotrichosis. Smears were reviewed for cytomorphological findings and were correlated with the histopathological findings. Positive cases were further subjected to culture for confirmation

Results: In our study, the sensitivity and specificity of FNAC in diagnosing lymphocutaneous sporotrichosis were 35% and 100% respectively. The sensitivity and specificity of biopsy in diagnosing lymphocutaneous sporotrichosis were 10% and 100% respectively. All seven cases diagnosed on FNAC were confirmed on fungal culture.

Conclusions: FNAC is an effective, useful and a minimally invasive procedure. FNAC is comparatively better than biopsy in detection of fungal spores in the diagnosis of lymphocutaneous sporotrichosis. With an experienced cytologist, it can be used routinely for the diagnosis for lymphocutaneous sporotrichosis.

Keywords: FNAC, Cytology, sporotrichosis, lymphocutaneous.

1. Introduction

Sporotrichosis is a granulomatous fungal infection caused by sporothrix schenkii which frequently causes lymphocutaneous lesions. Consequent to trauma, the fungus establishes itself in skin and subcutaneous tissue of gardeners, forestry workers, farmers, carpenters, and others who are involved in outdoor activities. The classic form is lymphocutaneous accounts for nearly 70% of cutaneous sporotrichosis cases. Lymphocutaneous form of sporotrichosis clinically presents with emergence of indurated papule, which progresses to nodule formation and ulceration. Isolated case reports describing the utility of Fine Needle Aspiration Cytology (FNAC) in the diagnosis of Lymphocutaneous sporotrichosis are available in the literature. [1-5] Sporotrichosis is the most frequently encountered subcutaneous mycosis in the sub-Himalayan belt.[6-8] Since Himachal Pradesh in India is a known endemic area for cutaneous sporotrichosis,[9] this study was conducted to assess the utility of FNAC in the diagnosis of lymphocutaneous sporotrichosis.

2. Material and methods

Fifty cases of clinically diagnosed cases of lymphocutaneous sporotrichosis who attended the Departments of Dermatology in a Medical College attached tertiary care center, Himachal Pradesh for a period of one year were studied. A brief history was taken and the patients were explained of the simple procedure of Fine Needle Aspiration Cytology (FNAC) and written consent was taken. FNAC was done under aseptic precautions. Nodules and indurated lesions were fixed in position between the

thumb and index finger of the free hand. A 23-25 gauge needle attached to disposable syringe fitted in to a detachable syringe holder (Franzen Handle) was passed in to the lesion and negative pressure was applied by retracting the piston. The needle was moved back and forth within the lesion different directions with negative pressure maintained. Needle was withdrawn after releasing the negative pressure. Two to three passes were adequate to obtain a satisfactory sample in most of the cases. Aspirated material was expressed on to glass slides and minimum of four smears were made. Smears were air dried and simultaneously stained with Giemsa and PAS stains. Smears were examined for the presence of characteristic fungal spores of *Sporothrix schenkii*. Biopsy of the lesion was also taken simultaneously and processed and stained by H&E and PAS stains. All samples were collected after taking written informed consent from the patients. Cytohistological correlations were done. Positive cases were further subjected to culture for confirmation. Fungal cultures were observed twice a week up to 30 days and morphological appearance of characteristic growth ascertained using micro-slide culture technique. Fungal culture was used as gold standard for calculating sensitivity and specificity of FNAC and skin biopsy in diagnosing lymphocutaneous sporotrichosis

3. Results and Observations

Patients' age ranged from 18 years to 55 years. Presentation with lesions on hands was most frequently seen in 32%, with arm (23%) and face (21%) in that sequence. In seven out of 50cases, FNA smears were positive for fungal spores. The cytologic findings in the positive smears showed large number of macrophages, few polymorphonuclear neutrophils and numerous round or oval, sometimes elongated, isolated and scattered yeast-like structures localized extracellularly or inside macrophages. These structures were more clearly visualized in PAS stained sections (Figure 1). Only 2 cases on biopsy revealed fungal spores which were confirmed with PAS stain. Both these cases were positive for fungal spores in FNAC smears also. Cultures from skin biopsy material of all the seven patients whose FNAC smears were positive for fungal spores, subsequently identified as *Sporothrix schenckii* as the causative organism. Culture of skin biopsy was positive for sporothrix schenkii in 20 cases out of 50 clinically diagnosed cases.

Statistical analysis of the data revealed 35% sensitivity, 100% specificity for FNAC as a diagnostic test for lymphocutaneous sporotrichosis. The sensitivity and specificity of biopsy in diagnosing lymphocutaneous sporotrichosis were 10% and 100% respectively.

Figure 1: PAS stained FNAC smears revealing macrophages, few polymorphonuclear neutrophils and round or oval, some isolated and scattered budding yeasts localized extracellularly or inside macrophages (PAS stain, 40x).

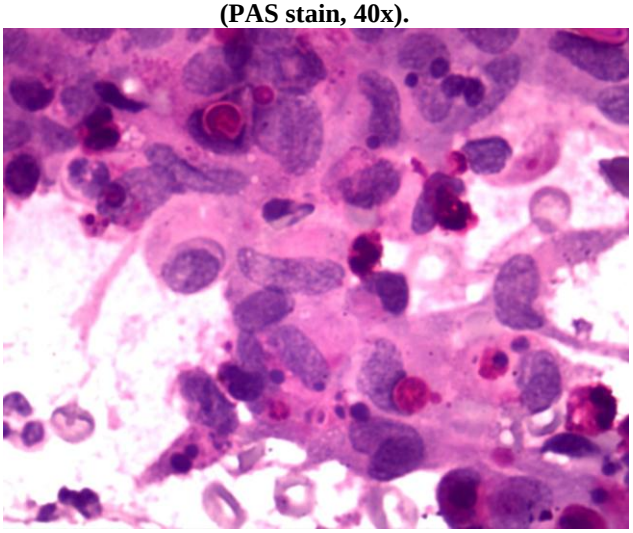
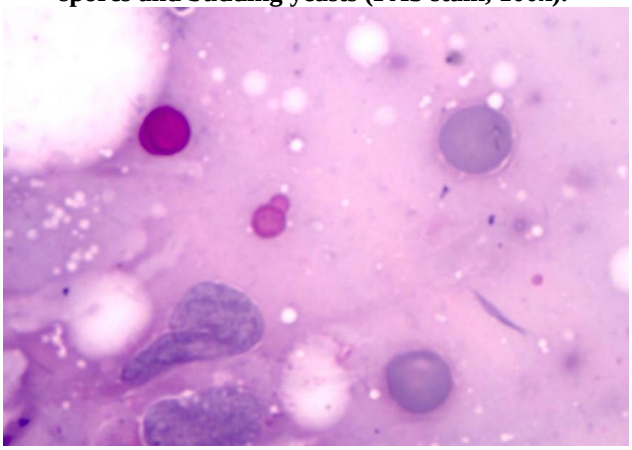


Figure 2: Higher magnification showing PAS positive spores and budding yeasts (PAS stain, 100x).



4. Discussion

Sporotrichosis is a form of chronic subcutaneous infection caused by Sporothrix schenckii. Sporotrichosis is classified into 4 clinical categories: (i) Lymphocutaneous, (ii) Fixed cutaneous, (iii) Multifocal or disseminated, and (iv) Extra-cutaneous. The most common clinical presentation is the lymphocutaneous variant, representing over 75% of all cases. [6] This variant is characterized by the emergence of indurated papule, which progresses to nodule formation and ulceration. Further nodules appear in the lymphatic course contiguous to the initial lesion and finally produce cutaneous fistulae. Biopsy of the nodule is long considered to be the best specimen for the definitive diagnosis. However the characteristic small narrow base budding yeast cells (2-5um) of *sporothrix schenkii* are often present in very low numbers and may be difficult to find.

Tissue sections should be stained using PAS digest, Grocott's methenamine silver (GMS) or Gram stain for the identification of the characteristic spores of sporothrix schenckii.

First case of lymphocutaneous sporotrichosis reported in the cytologic literature as diagnosed by FNA cytology was by Zaharopaulos in 1999. [1] They found Asteroid bodies and yeast cells with budding, highly suggestive of the disease in the cytologic and histologic preparations. Before this report, Farley *et al* [2] had described cytological features of *sporothrix schenkeii* in pulmonary cytology specimens. They recommended that Sporotrichosis should be considered in the differential diagnosis when small ovoid intracellular yeast cells (2 microns to 4 microns in length) with an apparent "halo" are observed within macrophages in pulmonary cytology specimens. Gerhard *et al* [4] have described the cytological features in a rare case of disseminated sporotrichosis. They were able to clearly visualize numerous round or oval, sometimes elongated, isolated and scattered yeast-like structures localized extracellularly or inside macrophages by Giemsa and Papanicolaou methods. In a recent report by Aggarwal *et al* [4] it was concluded that the FNAC of sporotrichosis is characteristic and can allow a confirmed diagnosis. Fontes *et al*[5] were able to identify chronic granulomatous inflammatory alterations and extracellular fungal structures consisting of periodic acid-Schiff-positive budding cells and spherical or elongated (cigar bodies) free spore forms in the exfoliative cytology specimen from the oral lesion of sporotrichosis in a HIV positive man.

The lymphatic nodules of lymphocutaneous sporotrichosis as well as the cutaneous nodules of multifocal systemic sporotrichosis at first show scattered granulomas within an inflammatory infiltrate; predominantly in the deep dermis and subcutaneous fat. These enlarge and coalesce to form irregularly shaped suppurative granulomata, and eventually a large abscess surrounded by zones of histiocytes and lymphocytes. In many instances, it is not possible to recognize the causative organisms of Sporothrix schenckii in tissue sections. [9] In many cases, negative findings are common even with diastase digestion of glycogen granules prior to staining of sections with the PAS reaction. Nor has staining with methenamine silver increased the frequency of positive findings in these cases. [10] Even in cases with positive findings, numerous sections often have to be examined before one or a few organisms are visualized. In our study also skin biopsy revealed low sensitivity (10%) in the detection of fungal spores. Even in cases with characteristic histological findings, numerous sections often have to be examined before one or a few organisms are visualized. In such cases, PAS stained FNAC smears may be of great value in finding the organism. Immunohistochemical staining using primary antibodies directed against S. schenckii may increase the percentage of cases in which the organism can be demonstrated to 83%, more than double that achieved with ordinary histochemical methods.[11] Though the histopathological findings in sporotrichosis is quite characteristic and may aid in the clinical diagnosis, fungal isolation by culture studies is confirmatory.

Our study revealed 35% sensitivity, 100% specificity, for FNAC as a diagnostic test in detection of fungal spores in lymphocutaneous sporotrichosis. The sensitivity and specificity of biopsy in finding characteristic fungal spores in lymphocutaneous sporotrichosis were 10% and 100% respectively. Thus FNAC proved to a better method for detection of fungal spores in the diagnosis of lymphocutaneous sporotrichosis. Extensive literature search did not reveal any such study for comparison.

In conclusion, FNAC is easier, less painful and more cost effective than the conventional biopsy in the diagnosis of lymphocutaneous sporotrichosis. PAS stained FNAC smears are more useful in the diagnosis of lymphocutaneous sporotrichosis than tissue biopsies. It is also concluded that cytologic findings of lymphocutaneous sporotrichosis is characteristic enough to allow a definitive diagnosis in clinically diagnosed cases.

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