

Evaluation of Fine Needle Aspiration Cytology versus Culture for Laboratory Diagnosis of Subcutaneous Mycosis

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ABSTRACT

Introduction: Subcutaneous swellings are commonly seen in clinical practice having varied pathology from epidermal inclusion cyst to malignancy. Subcutaneous mycosis is a fungal infection of subcutaneous tissue and dermis which is more prevalent in workers of rural, tropical, and subtropical regions. The subcutaneous infection mostly occurs due to minor trauma which goes unnoticed. Fine Needle Aspiration Cytology (FNAC) followed by microbiological analysis is a cost-effective and simple procedure and can be considered as one of the main modalities for early diagnosis. Moreover, it gives an early presumptive identification of fungus, which helps the clinician in a timely therapeutic approach.

Aim: To study the efficacy of FNAC in the diagnosis of subcutaneous mycosis in comparison with culture method.

Materials and Methods: A cross-sectional study, including clinically diagnosed cases of subcutaneous mycosis was evaluated between the periods of March 2019 to February 2021 in a tertiary care hospital in Northern Odisha. FNAC samples from all the cases of subcutaneous mycosis were studied cytologically and microbiologically. Cases were analysed and further evaluated on the basis of their age, sex, detailed clinical

history, and presentation. FNAC showed the presence of fungal elements in 20 cases. Microbiological evaluation of these samples was carried out.

Results: Out of the total 50 cases of subcutaneous mycoses, male (16): female (4) ratio was 4:1 and the commonest age group affected was 20-60 years of age (75%). The most common site of involvement was the lower limb (60%) followed by upper limb (30%). Almost all the cases were from rural areas with a history of trauma present in 11 cases (55%). Out of 50 clinically diagnosed cases, cytological examination revealed fungal element in 20 (40%) cases. The most common cytological diagnosis was abscess with neutrophilic infiltration seen in 13 cases (65%) followed by granuloma in 7 cases (35%). All the 20 samples were further evaluated in conventional methods like KOH mount and culture. Fungal agents were isolated in 13 (65%) cases. *Cladosporium* spp. was the commonest isolate in 5 cases (25%).

Conclusion: Subcutaneous mycosis is a rare disease. FNAC can be used as a rapid and cost-effective screening tool in the diagnosis of such cases as it is more sensitive. Though culture is the gold standard test, it is less specific.

Keywords: Abscess, Dematiaceous fungi, Fungal infection, Granuloma

INTRODUCTION

Subcutaneous mycoses are infections caused by saprophytic fungi, which get inoculated to the site of infection usually by penetrating injury. Such infections are more common in rural and agricultural populations [1]. Subcutaneous mycoses may be of various types like eumycetoma, chromoblastomycosis, phaeohyphomycosis, etc., [2]. As it usually presents with localised swelling without any obvious signs of inflammation, more often clinically it is mistaken for other benign lesions like an epidermoid cyst, sebaceous cyst, or cold abscess [3]. FNAC followed by histopathological examination is a principal diagnostic method required for the diagnosis of subcutaneous mycoses. It is reliable and less time-consuming than culture [4]. Though modern analysis including Polymerase Chain Reaction (PCR) is now available, it is time-consuming, costly and needs a special laboratory set-up. So, for early presumptive diagnosis, cytological evaluation of granulomatous lesions with or without giant cell reaction, necrotising inflammation with a careful search for fungal elements, histopathological examination, subsequent special stain, and culture to demonstrate and identify the fungus wherever possible provides indisputable evidence of invasive infections. The biopsy is painful and can cause scarring and requires more time and may be done in cases where the representative sample is not sufficient or correlation is required. Because of its morphological diversities, many of the fungi are seen under light microscopy by conventional diff-quick and hematoxylin and eosin (H&E) stain.

Special stains like gram stains, Periodic Acid Schiff (PAS), and Giemsa stains may be used for the identification of fungi in cytology specimens. Fungal elements are usually present in hyphae, budding yeast, endospore-forming spherules, or in combinations along with tissue reactions depending on their virulence [4].

Keeping in view the above background, the aim was to study demography, clinical presentation of subcutaneous mycoses, their diagnosis by FNAC and confirmation by culture isolation. As FNAC is a common and simple outpatient department procedure, it was used to diagnose subcutaneous mycosis. Biopsy was done in equivocal cases. Culture was used in the diagnosis and identification of the causative agent causing subcutaneous mycosis. The objective was to study the efficacy of FNAC in the diagnosis of subcutaneous mycosis.

MATERIALS AND METHODS

A cross-sectional study was conducted on clinically diagnosed cases of subcutaneous mycosis presenting as subcutaneous swellings in the department of pathology and microbiology in a tertiary care hospital in North Odisha from March 2019 to February 2021 with a total number of 50 cases.

Inclusion criteria: All cases of subcutaneous swellings clinically diagnosed as subcutaneous mycosis, reported in the section on cytopathology during the study period of March 2019-February 2021.

Exclusion criteria: Follow-up cases of subcutaneous swelling due to non infectious aetiology, malignancy, deep fungal infection.

IEC consideration and permission: The ethical practice of conducting research with IEC number 1 1/Dt. 19.06.2019 has been implemented.

Patient clinical data was collected from records maintained in cytology section and analysed with respect to age, sex, site of involvement, duration of the lesion, predisposing factors like thorn injury, and other comorbid conditions like diabetes mellitus, chronic non healing ulcers, high blood pressure and malignancy.

The subcutaneous swellings were examined. Most of them presented as a cystic mass and nodule. Patient was explained about the procedure of fine needle aspiration. A 23 G needle was inserted in the subcutaneous swelling and with a 10 mL syringe, aspiration was done. The aspiration material was usually fluid or pus and spread over a glass slide. In case of fluid aspiration of 2 ml, sample was centrifuged and from the deposits, smears were prepared. Two cytology smears were prepared in each case. One was kept for air drying and stained with Leishman and Giemsa stain. Another slide was kept for fixation by methanol and stained with H & E stain.

In the department of microbiology, all the samples were examined under direct microscopic examination in KOH mount and Gram staining. They were inoculated into blood agar, MacConkey agar, and two tubes of Sabouraud Dextrose Agar (SDA) with antibiotics incubated at 37°C & 25°C respectively. The growth in blood agar and MacConkey agar were examined after 24-48 hours and identified by conventional methods. Growth in SDA was examined every alternate day for the first week and thereafter twice a week for 6 weeks. The fungal growth was identified based on the time taken for growth to appear, colony morphology, and microscopic appearance.

The cytological features were studied. The equivocal cases sent for biopsy were traced from records and histopathological features were noted down. Special stains like PAS stain was done from the blocks of subcutaneous mycosis demonstrating fungus.

STATISTICAL ANALYSIS

Data analysis: Data entry was done using an MS Excel sheet. Categorical variables were expressed using frequency and percentages. Quantitative variables were expressed using the mean. Demographic data like age, sex, etc., were expressed in percentages. Hypotheses are formulated as follows.

H0: There is no significant difference between the effectiveness of both the tests.

H1: There is significant difference between the effectiveness of both the tests. The t-test was applied for hypothesis testing.

RESULTS

Out of the 50 cases, 20 (40%) were diagnosed to be subcutaneous mycosis [Table/Fig-1] and the majority were males (80%). The most common affected age group was between 20-60 years with a total number of 15 (75%) cases from this age group [Table/Fig-2]. The most common site of involvement was the lower limb in 12 (60%) cases followed by the upper limb in 6 (30%) cases and history of trauma in 11 (55%) of total cases [Table/Fig-3]. Almost all cases were from rural areas and were agricultural workers. There were no other comorbidities like diabetes mellitus, malignancies or human immunodeficiency virus (HIV) etc. Two cases had additional atypical presentations; one case presented with multiple erythema like lesions over the head & neck and upper arm and another case presented with multiple plaque-like lesions over the tongue. Abscess with neutrophilic infiltration and hyphae were the most common cytological features in 13 (65%) cases, followed by granuloma with giant cell reaction and hyphae in 7 cases in the subcutaneous mycosis cases [Table/Fig-3]. A

comparative analysis was done between different types of tests [Table/Fig-4]. [Table/Fig-5] demonstrated a patient who presented with a subcutaneous swelling over the ulnar border of right hand. The fungal structure was identified in H&E, Diff-Quick stain, and Gram stain. The most common fungal structure identified in this study was branching septate hyphae and most of them were dematiaceous [Table/Fig-6,7]. Histopathological examination was done in 6 equivocal cases (30%) out of 20 subcutaneous mycosis cases. Cases revealed non specific lymphohistiocytic infiltration, few giant cells and presence of branched, thin, septate hyphae intermingled with each other. PAS stain faintly demonstrated the fungal hyphae [Table/Fig-8].

Cases	Number of cases	Percentage of cases
Epidermoid cyst	15	30
Lipoma	5	10
Abscess	10	20
Fungal lesion	20	40

[Table/Fig-1]: Different cytological diagnosis in clinical subcutaneous mycosis (n=50).

Age	No. of cases	Male	Female
<20	1 (5%)	1 (5%)	0
20-60	15 (75%)	12 (60%)	3 (15%)
>60	04 (20%)	3 (15%)	1 (5%)
Total	20 (100%)	16 (80%)	04 (20%)

[Table/Fig-2]: Age and sex distribution of subcutaneous mycosis (n=20).

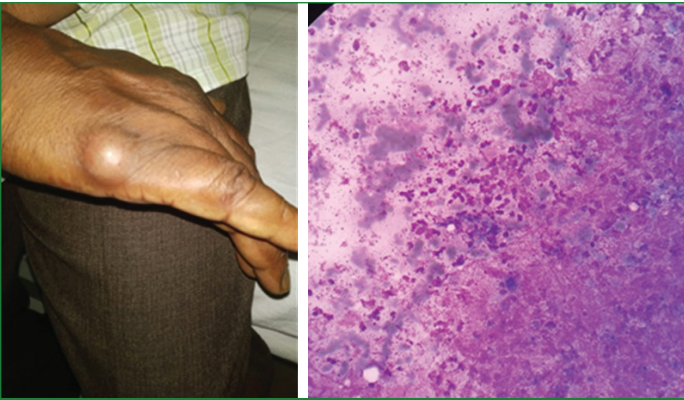
Sites of involvement	No of cases (n=20)	History of injury present	Cytological diagnosis	
			Abscess with fungal hyphae	Granuloma with fungal hyphae
Foot	9 (45%)	7 (35%)	4 (20%)	5 (25%)
Leg	3 (15%)	2 (10%)	2 (10%)	1 (5%)
Forearm	4 (20%)	1 (5%)	3 (15%)	1 (5%)
Wrist	2 (10%)	1 (5%)	2 (10%)	
Head and neck	1 (5%)		1 (5%)	
Multifocal	1 (5%)		1 (5%)	
Total	20 (100%)	11 (55%)	13 (65%)	7 (35%)

[Table/Fig-3]: Clinical data and cytological diagnosis in clinically suspected subcutaneous mycoses.

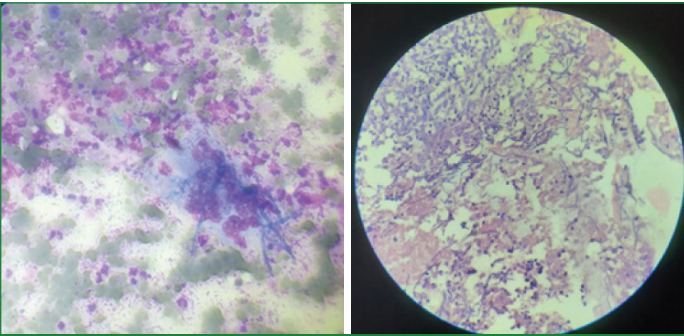
	Cytology (n=20)	Histopathology (n=6)	Culture (n=20)	
Neutrophilic abscess with fungal hyphae	13	04	Bacteria	Staphylococcal aureus without fungus 4 (20%)
Granulomatous inflammation with fungus	07	02	Fungal isolate	Cladosporium 5 (25%)
				Fonsecaea 3 (15%)
				Alternaria 2 (10%)
			Curvalaria 3 (15%)	
No growth			3 (15%)	

[Table/Fig-4]: Comparison between different types of examination in subcutaneous mycosis.

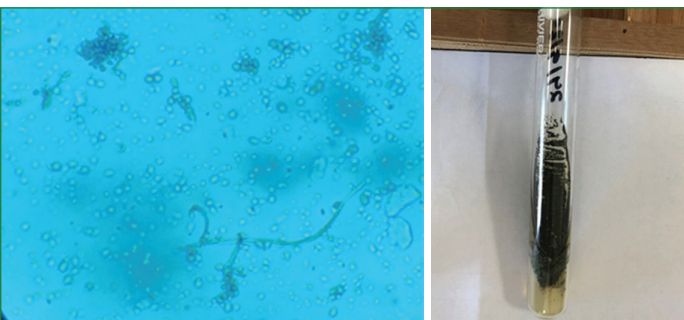
Out of 20 cases of cytologically diagnosed subcutaneous mycoses, fungal isolation was successful in 13 (65%) cases [Table/Fig-4]. Cladosporium species [Table/Fig-9,10] was the commonest followed by Fonscasea spp. and Curvalaria spp. Bacterial isolates like Staphylococcus aureus without any fungal element were found in 4 (20%) cases. There was no growth in 3 (15%) cases. May be fungal element had lost its viability either due to delayed transport to microbiology laboratory or the patient might have received treatment prior to collection of sample and fungal element could not be cultured. Staphylococcal infection was the superadded infection observed in 4 (20%) cases.



[Table/Fig-5]: Patient presented with a subcutaneous swelling over the ulnar border of right hand. **[Table/Fig-6]:** Photograph shows fungal hyphae with neutrophilic infiltration; H&E stain; 400X. (Images from left to right)



[Table/Fig-7]: Photograph shows fungal hyphae engulfed by a multinucleated giant cell with inflammatory cells like lymphocytes and neutrophilic infiltration; H&E stain; 400X figure. **[Table/Fig-8]:** Histopathology shows presence of branched, septate hyphae along with chronic inflammatory cells like lymphocytes and epithelioid cells, histiocyte and neutrophil (H & E, X400). (Images from left to right)



[Table/Fig-9]: Lactophenol cotton blue mount in high power showing septate brown hyphae with conidia of cladosporium spp. **[Table/Fig-10]:** Sabouraud Dextrose Agar (SDA) tube showing growth of Cladosporium spp. (Images from left to right)

The [Table/Fig-11] represents the result of the t-test applied and the outcome is 0.004729 which is less than the p-value of 0.05. Therefore, the null hypothesis is rejected and the alternate hypothesis is accepted that there is a strong significant difference between test methods (cytology and culture).

	Variable	Variable
Mean	1	0.65
Variance	0	0.239474
Observations	19	20
Hypothesised mean difference	0	
df	19	
t Stat	3.198557	
P (T<=t) one-tail	0.002365	
t Critical one-tail	1.729133	
P (T<=t) two-tail	0.004729	
t critical two-tail	2.093024	

[Table/Fig-11]: Statistical analysis between FNAC and culture.

DISCUSSION

Pathogenic fungi or mycosis may infect various tissues ranging from skin to subcutaneous tissue and may disseminate to deeper organs like brain, heart, lungs and liver. The term subcutaneous mycosis describe the lesion limited to dermis and subcutaneous tissue and elicit a chronic, localised inflammatory response at the inoculation site of the aetiological agent.

Subcutaneous mycoses cases are mostly seen in the hot and humid climate of the tropical region. In Odisha, the geographically northern part is a hilly tropical region with more forest land where the subcutaneous fungal infection is a common occurrence. We have studied 50 cases within 2 years. Verma S et al., also reported 70 cases over 4 years in the Himalayan region [3]. Maybe the number of cases is under-reported because of the callousness of the people due to which they do not take such lesions seriously and many going for indigenous methods of treatment. The primary fungal infection occurs due to traumatic penetration. Usually, this traumatic injury occurs in the barefooted population of the world, agricultural workers, mountain hikers, and gardeners [4]. Nearly all other studies showed involvement mostly of lower limbs. Most of our patients were forest dwellers or living in villages adjoining forests whereas Yahya S et al., demonstrated agricultural occupation as a risk factor [5].

Our study demography revealed most patients were in middle age group (75%). Micro-trauma leads to implantation of fungi in the subcutaneous tissue leading to chronic inflammatory tissue reaction. History of minor trauma was found in 55% cases in hard-working daily labourers whereas others could not recall.

Clinically, most of the patients of subcutaneous mycoses present with cystic swelling, plaque like lesion, ulcers and warty verrucous growth [5]. In present study, all 20 cases of subcutaneous mycoses cases presented as cystic or solid subcutaneous swellings. Cystic swelling was the commonest presentation in other studies too [6-9]. Carrion in 1950, described five different clinical forms of Chromoblastomycosis presenting as nodule, tumorous, verrucous, cicatricial and plaque type.

Most of the fungi produce hyphae- septate or aseptate, arranged as branched intertwined hyphae. Dimorphic fungi exist both in yeast and hyphae form depending upon the environmental condition. In the present study, the distinguishing parameters to differentiate between different fungi were based on budding, presence of capsule, septa and branching pattern.

Cytological evaluation of all the aspirated samples showed the presence of fungal elements in 20 cases (40%). There are very few studies on FNAC of the samples of subcutaneous mycosis for correlation [10,11]. Most of the studies were carried out on biopsy and culture.

Fungal hyphae can induce either granulomatous inflammation or suppurative abscess [12,13]. On aspirated material, H&E and Giemsa staining demonstrated the presence of fungal hyphae as branched, parallel hyphae with or without septation and most were dematiaceous. The background contained polymorphs and histiocytes in suppurative lesions and granuloma lesions featured multinucleated giant cells, eosinophils and lymphocytes. Verma S et al., and Priyadarshini G et al., demonstrated giant cell reaction with granuloma as their most common finding on histopathological examination [3,10]. Subhashini R and Bhat RV observed heavy eosinophilic infiltration in 36% of cases [4].

This study revealed Cladosporium spp. (25%) as the most common fungal isolate followed by Fonsecaea spp. and Curvularia spp. (15% each) whereas others isolated Phialophora spp. and Fonsecaea spp. as the predominant isolate [Table/Fig-12]. Verma S et al., found chromoblastomycosis as the commonest organism causing subcutaneous mycosis [3] Yahya S et al., observed subcutaneous mucormycosis as the commonest case followed

Yahya S et al., (2016) [5]	Rachel E et al., (2017) [12]	Verma S et al., (2018) [3]	Subhashini R and Bhat RV (2019) [4]	Present study
Basidiobolus (Mucormycosis) (31%)	Cladosporium (33%)	Fonseca (48%)	Exophiala (Phaeohyphomycosis) (68%)	Cladosporium (25%)
Phialophora (Chromoblastomycosis) (18%)	Aspergillus (33%)	Sporothrix (41%)	Hyalohyphomycosis (27%)	Fonseca (15%)
Eumycetoma (25%)		Cladosporium (7%)	Chromoblastomycosis (4.5%)	Curvularia (15%)

[Table/Fig-12]: Isolation of different type of fungus in comparison with other studies.

by chromoblastomycosis. Culture revealed *Basidiobolus ranarum* in mucormycosis and *Phialophora* in chromoblastomycosis as the commonest aetiological agent [5]. All the fungal isolates were dematiaceous fungi; hence 65% of the subcutaneous mycoses cases were diagnosed as phaeohyphomycosis in the present study. Subhashini R and Bhat RV also identified phaeohyphomycoses as the commonest type of subcutaneous mycosis. Priyadharshini G et al., found phaeohyphomycosis as the only causative organism in their study [4, 10].

Elephantiasis and lymphedema are some rare complications associated with chromoblastomycosis due to chronic inflammation followed by fibrous scarring. Verma S et al., also reported squamous cell carcinoma as a long standing complication of chromoblastomycosis [3].

While analysing the efficacy of FNAC in the diagnosis of subcutaneous mycosis, 20 cases showed presence of fungal element whereas culture method isolated fungus in 13 cases. There is significant statistical difference between the tests. FNAC was found to be sensitive and can be used as a screening procedure. Culture, the conventional method of diagnosis is specific, but less sensitive as viable fungus presence is essential for the growth. All the cases of subcutaneous mycosis were followed-up and they had shown improvement after taking antifungal drugs like Itraconazole for 3 months.

Limitation(s)

Histopathological study could not be done in all the cases as it was an invasive procedure.

CONCLUSION(S)

As FNAC is more sensitive, it is a useful tool for early diagnosis of subcutaneous mycosis as it is cheap and less time-consuming in experienced hands. Subcutaneous mycosis is usually more prevalent in hilly forest areas with a tropical climate and as this region has the same topography; more screening is required

along with awareness among the natives. The present study was done to assess diagnostic efficacy of FNAC in the detection of subcutaneous mycosis in a new medical college in Northern Odisha with limited resources.

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