



Original Research Article

Mycological profile of subcutaneous skin biopsy samples in suspected subcutaneous mycosis, Are we missing some fungi? A tertiary care hospital based study

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ABSTRACT

Introduction: Subcutaneous mycosis includes a heterogeneous group of fungal infections that develop at the site of transcutaneous trauma. A rise in surviving population of individuals with co-morbidities together with lapses in infection control practices especially in rural India has led to emergence of myriad species of fungi causing such infections in recent years. This poses a diagnostic challenge to the clinicians. We therefore felt a need to study the mycological profile of subcutaneous tissue biopsies suspected of fungal etiology in our hospital setup.

Material and Methods: This was a Retrospective cross-sectional observational study conducted for a period of 4 years from Jan 2015 to Jan 2019 in the Department of microbiology, Government medical College Srinagar. A total of 148 specimens of subcutaneous tissue biopsies of patients suspected of localized fungal Infection were processed by microscopy and culture for fungi.

Results: Among a total of 148 subcutaneous tissue biopsy samples 76 (51%) were positive for fungi on KOH and culture. Mycological profile revealed that majority of fungi were Dermatophytes (n=22; 29%) and Yeasts (n=21; 28%). Third most common causative fungi isolated were Chromoblastomycetes (n=8; 11%), Phaeohyphomycetes (n=8; 11%) and Aspergilli (n=8; 11 %) comprising, A.fumigatus (n=11) and A.terreus (n=03), followed by Hyalohyphomycetes (n=4; 5%), sporotrichosis (n=3; 4%) and Zygomycetes (n=4 ;5.3%).

Conclusions: Dermatophytes and yeasts are emerging subcutaneous fungal infections. Active surveillance for all types of fungal infections should be done at all levels to address knowledge gaps that exist in etiology, pathogenesis and manifestations of fungal diseases of skin and subcutaneous tissue.

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1. Introduction

Subcutaneous mycoses also known as implantation mycoses, refers to the fungal infections of skin, subcutaneous tissue and bones caused by inoculation of saprophytic fungi that lead to progressive local disease and tissue destruction.^{1,2} It includes a heterogeneous group of fungal infections that develop at the site of transcutaneous trauma. Infection slowly evolves as the

etiologic agent survives and adapts to the adverse host tissue environment. Traditionally, types of subcutaneous mycoses seen are sporotrichosis, mycetoma, chromoblastomycosis; Phaeohyphomycosis, Hyalohyphomycosis, lobomycosis, rhinosporidiosis, and subcutaneous Zygomycosis.³ Such infections can be present in both immunocompromised and immunocompetent individuals. A rise in surviving population of individuals with co-morbidities together with lapses in infection control practices especially in rural India has led to emergence of myriad species of fungi causing

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such infections in recent years.⁴ Diagnosis of subcutaneous mycosis can sometimes be challenging, for example primary cutaneous presentations of Phaeohyphomycosis in affected persons may be misdiagnosed as cutaneous tumors due to the long duration of presenting symptoms.⁵ This has made the microbiological culture and identification of subcutaneous lesions even more relevant. We therefore felt a need to study the mycological profile of subcutaneous lesions suspected of fungal etiology in our hospital setup.

2. Materials and Methods

This was a Retrospective cross sectional observational study conducted for a period of 4 years from Jan 2015 to Jan 2019 in the Department of microbiology Government medical College Srinagar.. A total of 148 specimens of subcutaneous tissue biopsies of patients suspected of localized fungal Infection sent from Dermatology OPD of government SMHS hospital in this period were analyzed. The study was approved by the institutional ethical committee. A Proper written and informed consent along with detailed history of the patients pertaining to age, sex, occupation, prior treatment, and site of lesions was taken before taking the samples and were referred to our Microbiology laboratory for further investigations. Each specimen was collected under aseptic precautions. The samples were kept in a sterile gauze piece in a sterile container and transported to our laboratory within 2 hours.

2.1. Microbiological processing

All samples were handled in BSL (biosafety level) II cabinets and processed using standard procedures.⁶ Direct microscopy: Samples were cut to a size of 1-1.5 mm smaller pieces with sterile scissors. A piece was subjected to 20% potassium hydroxide (KOH) and examined for fungal elements under low (10 x) and high power (40 x) magnification. Culture and identification: Pieces of the sample were implanted on the Sabouraud's dextrose agar containing chloramphenicol and Gentamicin, Dermatophyte test medium (Himedia) and Potato dextrose agar (Himedia). The inoculated specimens were incubated at 30°C for 4 weeks and checked every day for 1st week and then twice a week in 2nd, 3rd and 4th week for fungal growth Mold-form fungi were identified using colony morphology, microscopic findings and slide culture technique. Microscopic morphology of the growth was examined with lacto phenol cotton blue (LPCB) stain for final identification. The Yeast-form fungi were identified according to macroscopic morphology, LPCB mount, Gram staining, Germ tube test, Chromagar identification and Dalmau plate technique.

3. Results

Among a total of 148 subcutaneous tissue biopsy samples 94 (63.51%) were of male and 54 (36.48%) were of female. Majority of the cases were of the age group 15 - 30 yrs which accounted for 86 cases (58.10%) (Table 1). Depending upon the site of lesion involved, majority of cases had their lower limbs involved which accounted for 94 positive cases (63.51%) followed by lesions on upper limb accounting for 32 cases (21.62%) least no of cases (08) had multifocal lesions (5.40%). (Table 2). Out of 148 samples of subcutaneous tissue, 113 were found to be positive on KOH microscopy for fungal elements out of these 76 were culture positive. Out of 35 samples positive on KOH microscopy 22 grew fungi on culture. Specimens positive on KOH and fungal culture both, which were 76 in number, were considered to be significant causative agents of subcutaneous mycosis in our study. (Table 3) Mycological profile of the seventy six, both culture and direct KOH positive specimens showed that majority of fungi were Yeasts (n=21; 28%) and Dermatophytes (n=22; 29%). The other common causative fungi isolated were Chromoblastomycetes (n=8 ; 11%) Phaeohyphomycetes (n=8; 11%) and Aspergilli (n=8; 11%) comprising, *A.fumigatus* (n=11) and *A.terreus* (n=03), followed by Hyalohyphomycetes (n=4; 5%), sporotrichosis (n=3;4%) and Zygomycetes (n=4 ;5.3 %). (Table 4)

4. Discussion

Subcutaneous mycoses are fungal infections that primarily involve the dermis and subcutaneous tissues and rarely disseminate. They are characterized by papulo-nodules, verrucous hyperkeratosis or ulcerated plaques, cysts, abscesses, non-healing ulcers or sinuses. Prevalent in the tropical and subtropical regions with hot and humid climate. These infections are more common in the adult males from the rural areas who are predisposed to trauma and contact with soil. In the present study, the frequency of suspected subcutaneous fungal infections was more in males, which is due to the fact that they were more exposed to outdoor working environment. The ratio of male to female was 2.7:1 which was in accordance to the earlier studies by Kindo et al.⁷ and Yahya et al.⁸ who reported a ratio of 3:1. The most common age group affected was 15-30 years (58.10%) and the commonest occupation associated with the presentation was agriculture/farming (39.18%) in concordance with 52.8% reported by Yun et al.⁹ Specimens received most commonly were from the lower extremities (63.51%) similar to the observation of 64% lower limb involvement by Bhat et al.¹⁰ and 68.3% by Sivayogana et al.¹¹ showing that Occupational exposure represents important risk factor for subcutaneous mycoses, especially on trauma-prone sites such as lower limb. The underlining diseases associated with these infections in this study were diabetes mellitus

Table 1: Prevalence of subcutaneous mycosis according to age group

Age group	No. of patients	Percentage (%)
< 15	28	18.91
15-30	86	58.10
30-60	34	22.97

Table 2: Distribution according to the site of lesion involved

Site involved	No. of positive cases	% age
Lower limb	94	63.51
Upper limb	32	21.62
Neck and face	14	9.45
Multifocal lesions	8	5.40

Table 3: Distribution of samples according to the culture and KOH positivity

Total cases	KOH + Culture +	KOH – Culture +	KOH – Culture -	KOH + Culture -
148	76	22	23	27

Table 4: Spectrum of fungi isolated from lesions suspect of subcutaneous mycosis

	Species	Frequency (number of isolates)	Percentage
1. Yeasts	<i>Sporothrix schenckii</i>	3	21 (28%)
	<i>Candida albicans</i>	12	
	<i>Non-albicans Candida</i>		
	<i>Candida kefyr</i>	1	
	<i>Candida tropicalis</i>	3	
	<i>Candida guilliermondi</i>	2	
2. Dermatophytes	<i>Trichophyton. rubrum</i>	12	22 (29%)
	<i>Trichophyton. mentagrophyton</i>	10	
3. Phaeohyphomycosis	<i>Exophiala spp.</i>	4	8 (11%)
	<i>Cladophialophora spp.</i>	4	
4. Hyalohyphomycosis	<i>Paecilomyces lilacinus</i>	1	4 (5%)
	<i>Fusarium spp.</i>	1	
Aspergillosis	<i>Scopulariopsis breviculis</i>	3	8 (11%)
	<i>Aspergillus fumigatus</i>	5	
	<i>Aspergillus. terreus</i>	3	
5. Chromoblastomycosis	<i>Phialophore spp.</i>	3	8 (11%)
	<i>Cladosporium carrionii</i>	5	
6. Zygomycosis	<i>Rhizopus arrhizus</i>	3	4 (5.3%)
	<i>Mucor spp.</i>	1	
Total		76	

(21.62%) followed by malignancies (14.18%), Sivayogana et al.¹¹ has also reported diabetes mellitus to be the most common co-morbidity.

Fungal culture profile, in the present study showed a peculiar observation that majority of the specimens were positive for yeast and Dermatophytes in both culture and microscopy. Since histopathological correlation of samples was not done, many questions regarding such findings arise. Misdiagnosis of superficial mycosis for subcutaneous mycosis by junior staff in OPD and subsequent biopsy samples yield Dermatophytes or yeasts? Mimicking of clinical presentation of subcutaneous mycosis by agents that usually cause superficial infections? Are superficial lesions

penetrating to subcutaneous lesions owing to increasing drug resistance and immunocompromised nature of subjects?. The answers to these questions need to be sought. Among yeasts *C. albicans* was the predominant pathogen isolated. Subcutaneous candidiasis a rare disease entity however there have been few case reports of multiple¹² and solitary subcutaneous *Candida* lesions^{13–16} throughout the world. *Trichophyton rubrum* and *Trichophyton mentagrophytes* were the common Dermatophytes isolated. There could be a possibility of deep dermatophytosis like picture caused by these fungi. Studies^{17,18} have shown that deeper dermis and subcutaneous dermatophyte infections can occur in patients with compromised immune

systems, solid organ transplantation, hematological malignancy, immunosuppressive therapy, or congenital immune deficiency.^{17,18} In a recent review on emerging atypical and unusual presentations of Dermatophytes in India, Dorga and Narang⁴ have highlighted this growing trend of atypical dermatophytosis in India.

Common Hyalohyphomycetes or non-dematiaceous molds reported to cause isolated cases of subcutaneous infections are *Fusarium*,^{19,20} *Paecilomyces*,^{21,22} *Acremonium*,^{23,24} *Scopulariopsis*.²⁵ They commonly present in the tissue form as colourless (hyaline) septate fungal hyphae with no pigment within the walls. Although saprophytes, hyalohyphomycetes are emerging as fungi causing varied type of infections owing to changing scenario of healthcare world over, we isolated hyalohyphomycetes from five percent (n=4) of culture positive skin biopsy tissue samples. Bordoloi et al.²⁶ in a series of 15 cases of histopathologically confirmed subcutaneous mycosis from north-east India grew hyalohyphomycetes in three samples (20%). These fungi are known to cause abscesses, cysts or tumor like lesions after traumatic implantation. Although more prevalent in immunocompromised patients deep mycosis with hyalohyphomycetes may also present as subcutaneous lesions.²⁷

We know that *Aspergillus* spp are distributed throughout the environment in soil, water, air and are opportunistic pathogens causing infection at various body sites Primary cutaneous aspergillosis usually involves sites of skin injury, namely, at or near intravenous access catheter sites, at sites of traumatic inoculation, and at sites associated with occlusive dressings, burns, or surgery. In the recent times, there have been case reports of subcutaneous Aspergillosis. Findings in our study show eleven percent culture positive skin biopsy samples growing aspergilli. This observation guides to the fact that non pulmonary aspergillosis should not be overlooked²⁷ and thoroughly investigated.

Among the traditionally perceived causes of subcutaneous mycosis, Chromoblastomycetes (n=8; 11%) and phaeohyphomycetes (n=8; 11%) were more frequently isolated than *sporothrix schenckii* (n=4; 3%) in our specimens. Bordoloi et al.²⁶ Verma et al.^[32] in a study of few confirmed cases of subcutaneous mycosis have shown that chromoblastomycosis is more common in north-east India than sporotrichosis. Bhat et al.¹⁰ also reported a higher prevalence of chromoblastomycosis in subcutaneous mycosis in coastal karnataka.

5. Conclusion

The mycological profile of subcutaneous tissue biopsy samples in our study has shed new light into the evolving epidemiology and etiology of fungal infections. Yeasts and dermatophytes can be isolated from subcutaneous tissue biopsy lesions and such finding should not be overlooked by clinicians. We believe active surveillance for all types

of fungal infections should be done at primary, secondary and tertiary healthcare levels. This will address many knowledge gaps that exist in the etiology, pathogenesis and manifestations of fungal diseases of skin and subcutaneous tissue.

6. Conflict of Interests

The authors have no conflict of interest to declare.

7. Source of Funding

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