Epidemiology of Subcutaneous Mycoses in Northeast India: A Retrospective Study

Shikha Verma, Binod Kumar Thakur, Vandana Raphael¹, Devinder Mohan Thappa

Abstract

Background: Subcutaneous mycoses, although rare, are frequently reported from northeast India. Their spectrum varies with geographic region. Materials and Methods: We evaluated clinical records and histopathological features of subcutaneous mycoses cases seen during April 2013 to March 2017. Results: A total of 70 patients (44 males and 26 females) of subcutaneous mycoses were analyzed. Sixty-one percent of patients were 20–60 years old. Duration of the disease ranged from 3 months to 25 years. Most common site of involvement was the lower limb (32, 46%), followed by the upper limb (25, 36%). A history of trauma was obtained from 76% of patients. Eighty-seven percent of patients were from rural area. Ninety-two percent of patients were agricultural workers. There were 30 established cases of chromoblastomycosis and 16 cases of sporotrichosis. In 24 cases, subcutaneous mycosis was suspected clinically and showed some improvement to empirical itraconazole therapy. Multifocal lesions were seen in six patients. Complication of subcutaneous mycoses in the form of invasive squamous cell carcinoma was seen in one patient. On histopathological examination, verrucous hyperplasia was seen in 93% of cases. Granulomas with suppuration were seen in 77% of cases and granulomas without suppuration were seen in 14.3% of cases. Copper penny bodies were appreciated in 42.8% of cases. Fungal culture was positive only in 55.7% of cases. There was growth of Sporothrix schenckii in 16 patients, Fonsecaea sp. in 19, Cladosporium sp. in 3, and Curvularia sp. in 1. Conclusion: Chromoblastomycosis was the most common subcutaneous mycoses seen in northeast India followed by sporotrichosis. The diagnosis remained a challenge in a few cases as the culture positivity was very low. Suppurative granulomas in histopathology played a corroborative role. Therapeutic trial of itraconazole for 2 months was worth trying in such cases.

Key Words: Chromoblastomycosis, northeast India, sporotrichosis, subcutaneous mycoses

Introduction

Subcutaneous mycoses are the infections caused by the fungi present in the natural environment that are directly inoculated into the dermis or subcutaneous tissue through a penetrating injury from vegetative materials.¹ They affect population in rural communities, often in humid, tropical, or subtropical regions of developing countries. The various types of subcutaneous mycoses are sporotrichosis, mycetoma, chromoblastomycosis, phaeohyphomycosis, lobomycosis, rhinosporidiosis, and subcutaneous zygomycosis. Subcutaneous mycoses are associated with significant morbidity.² Misdiagnosis of cutaneous lesions is common. Histopathological examination is the principal investigation required to diagnose subcutaneous mycoses. It is reliable and less time-consuming as compared to cultures.³ However, discrepancies between histopathology and culture results are sometimes seen, with resultant treatment delays and increased morbidity. Molecular diagnostic techniques such as polymerase chain reaction (PCR) are now available at some centers for diagnoses of Fonsecaea sp. We herewith report series of cases seen in our tertiary care center from 2013 to 2017.

Materials and Methods

We reviewed clinical records and histopathological features of subcutaneous mycoses in cases from April
2013 to March 2017 after obtaining due permission from the Institutional Ethics Committee. Detailed analysis of these cases was done with special reference to history, clinical examination, mycological culture, histopathology, and periodic acid–Schiff (PAS) staining of biopsies from the cutaneous lesions. A total of 70 patients of subcutaneous mycoses were finally evaluated according to age, gender, profession, origin, location of lesions, number of lesions, histopathological characteristics, prior treatments, etc. In each case, two punch biopsies of size 5 mm were taken. One was sent in formalin for histopathological examination. The other one was sent in normal saline for fungal culture. Sabouraud dextrose agar was used as culture medium. Average time taken for positive growth was 4 weeks.

Results

**Patient characteristics and clinical findings**

A total of 70 immunocompetent patients (44 males and 26 females) of subcutaneous mycoses were evaluated. Male:Female ratio was 1.7:1. Forty-three (61%) patients were aged between 20 and 60 years. However, 14 (20%) cases were <20 years while 13 (18.5%) cases were >60 years old. Duration of the disease ranged from 3 months to 25 years. Most common site of involvement was the lower limb seen in 32 (46%) cases, followed by the upper limb in 25 (36%) cases, head and neck in 12 (18%) cases, and trunk in 1 (2%) case [Figures 1-5]. A history of trauma was obtained in 53 (76%) cases. Sixty-one (87%) cases were from rural areas. Sixty-five (92%) patients were agricultural worker [Table 1].

A definitive diagnosis of chromoblastomycosis could be made only in 30 patients and sporotrichosis in 16 patients where fungal elements were seen either in histopathology or in fungal culture. However, there were 24 more clinicopathologically suspected cases of subcutaneous mycoses, which showed improvement to 2 months empirical itraconazole therapy of 100 mg twice daily. Multifocal lesions were seen in six patients. Complication of subcutaneous mycoses in the form of invasive squamous cell carcinoma [Figure 6] was seen in one patient, in whom chromoblastomycosis lesions on feet were present for 20 years. Among 16 cases of sporotrichosis, 9 were fixed cutaneous type. A case of sporotrichosis mimicking multiple ecthyma was also seen [Figure 4]. In our series, 14 patients were misdiagnosed as cutaneous tuberculosis elsewhere and received antitubercular therapy (ATT).

**Histopathological characteristics**

Verrucous hyperplasia was seen in 65 (93%) of 70 cases and epidermal thinning in 3 cases. Two biopsies did not include epidermis. Suppurative granulomas were seen in 54 (77%) cases and tuberculoid granulomas were seen in 10 (14.3%) cases [Figures 7 and 8]. These granulomas were mainly situated at the dermoepidermal junction. Six (8.5%) cases showed nonspecific lymphohistiocytic infiltrate in upper dermis. Numerous eosinophils and eosinophilic abscesses were seen in 57 (81.4%) cases. The rest 13 cases showed scant eosinophils. Foreign body giant cells were seen in 46 (66%) cases. Copper penny bodies were appreciated in only thirty cases [Table 2]. None of the biopsies showed caseation and all were

![Figure 1: Verrucous plaques on dorsum of foot: Chromoblastomycosis](image)

| Table 1: Clinical findings |
|---------------------------|
| **Patient characteristics** | Number (%) |
| Age (years) | |
| <20 | 14 (20) |
| 20-60 | 43 (61.4) |
| >60 | 13 (18.6) |
| Sex | |
| Male | 44 (62.86) |
| Female | 26 (37.14) |
| Disease duration | 3 months-25 years |
| History of trauma | 53 (76) |
| Rural areas | 61 (87) |
| Site involved | |
| Lower limb | 32 (46) |
| Upper limb | 25 (36) |
| Head and neck | 12 (18) |
| Multifocal lesions | 6 (8.6) |

| Table 2: Histopathological findings |
|------------------------------------|
| **Histopathological characteristics** | Number (%) |
| Epidermal verrucous hyperplasia | 65 (93) |
| Granulomas | 64 (91.4) |
| Suppurative granulomas | 54 (77) |
| Tuberculoid granulomas | 10 (14.3) |
| Eosinophilic abscesses | 57 (81.4) |
| Foreign body giant cells | 46 (66) |
| Copper penny bodies | 30 (42.8) |
negative for acid-fast bacilli. PAS positivity was seen in 42.8% of cases.

**Microbiological findings**

Mycological cultures were positive in 39 (55.7%) cases. There was growth of *Sporothrix schenckii* in 16 patients, *Fonsecaea* sp. in 19, *Cladosporium* sp. in 3, and *Curvularia* sp. in 1.

**Discussion**

Subcutaneous mycoses are often reported from sub-Himalayan region and northeast India.[1] We have seen 70 patients of subcutaneous mycoses over a period of 4 years. This may be attributed to the high presence of these fungi in the hot and humid environment of mountainous regions of the northeast India.

Primary subcutaneous fungal infections are most commonly caused by traumatic implantation of the vegetative material containing the fungi into the skin.[2] Most of our patients were middle aged, male, and agricultural worker of rural areas of northeast India including Meghalaya, Nagaland, Mizoram, and Assam. Most of them had a primary subcutaneous mycosis on lower limb. There were no systemic signs or symptoms. Occupational exposure represented important risk factor for subcutaneous mycoses, especially on trauma-prone sites such as lower limb and hand. Male predominance in our series was attributed to the outdoor working conditions to which men were exposed. These findings were similar to most of the previous studies.[1-4] However, a history of trauma was not obtained in all the cases (76% in our series) as the patients might not notice it during strenuous outdoor physical activities.

Chromoblastomycosis, which presents as verrucous plaques and nodules, is caused by a variety of dematiaceous fungi, e.g., *Fonsecaea pedrosoi* and *Phialophora verrucosa*. In our series, thirty cases showed copper penny bodies on histopathological examination and were diagnosed as chromoblastomycosis. However, positive fungal culture was seen in only 23 of these cases. We observed multifocal lesions of chromoblastomycosis...
Verma, et al.: Subcutaneous mycoses

inoculation might occur simultaneously through a single episode of trauma. If one lesion was located far from the other, there was a possibility of fungal metastasis via a circulatory route. Well-known complications of chromoblastomycosis included associated infections leading to lymphedema and elephantiasis. There had been reports that prolonged progression and the presence of chronic inflammation and fibrous scarring might predispose to malignant neoplasms such as malignant melanoma and squamous cell carcinoma. In our series, one case of long-standing chromoblastomycosis of foot was complicated by squamous cell carcinoma. There had been few reports of this complication in chronic lesions of chromoblastomycosis.

Sporotrichosis is a chronic infection caused by S. schenckii, which is commonly found on senescent vegetation in humid areas. Lymphocutaneous type is the most common type of sporotrichosis. However, we observed a higher number of fixed cutaneous type (56% cases) of sporotrichosis. A patient with sporotrichosis on the face was seen, which was an unusual site. In our study, a definitive diagnosis of sporotrichosis could be made only in 16 patients where fungal cultures were positive. However, in all these cases of sporotrichosis, histopathological features showed suppurative granulomas, but asteroid bodies could not be appreciated.

The diagnosis of subcutaneous mycoses can be challenging in many cases. The clinical lesions as well as histopathological features may be very similar to other granulomatous diseases of the skin. Many of these cases can be misdiagnosed. Some lesions may show epithelioid granulomas, no fungal elements, and negative fungal cultures. In our study, 14 such patients of subcutaneous mycoses were misdiagnosed as cutaneous tuberculosis elsewhere and received ATT without any improvement. Sometimes, only suppurative granulomas are seen in histopathology, with no fungal element and negative fungal culture. There were 24 clinically suspected cases of primary subcutaneous mycoses in our series where definitive diagnosis of subcutaneous mycoses could not be made. All these patients showed suppurative granulomas in histopathology, but fungal elements could not be demonstrated. These patients showed some improvement after 2 months of empirical itraconazole therapy of 100 mg twice daily. We suggest that a 2-month therapeutic trial of itraconazole can be tried in difficult cases of suspected subcutaneous mycoses. Histopathological features of cutaneous lesions correlate with the slow adaptation of the fungi to the adverse host tissue environment. A granulomatous inflammatory pattern is the most commonly described histopathologic feature. Two types of tissue reactions are described:

(a) a granulomatous reaction with a suppurative granuloma

Verma, et al.: Subcutaneous mycoses

Figure 6: Fleshy mass of squamous cell carcinoma growing over a plaque of chromoblastomycosis

Figure 7: Histopathology showing foci of multiple suppurative granulomas consisting of epithelioid cells, lymphocytes, neutrophils, histiocytes, and copper penny bodies (H and E, ×200)

Figure 8: Histopathology showing multiple epithelioid granulomas with Langhans giant cells (H and E, ×400)
with several fungi cells in the cutaneous lesion presenting as verrucous plaque and (b) a granulomatous reaction with a tuberculoid granuloma with few fungus cells in the cutaneous lesion presenting as atrophic plaque. It was suggested that patients with skin lesion presenting with verrucous plaque had a Th2 immunological response, while patients with skin lesion presenting with erythematous atrophic plaque had a Th1 response. Uribe et al.,[17] described the granulomas modified by the presence of polymorphonuclear neutrophils as the organized mixed mycotic granulomas which were seen in 88.46% of the cases of chromoblastomycosis. An equal proportion of cases which exhibited pseudoepitheliomatous hyperplasia were also noted by them, with the epithelium playing an important role in the transepidermal elimination of the fungus.[17] We appreciated epidermal verrucous hyperplasia in 93%, suppurative granulomas in 77%, and tuberculoid granulomas in 14.3% cases.

Skin tissue culture may fail to show fungal growth in all the cases of subcutaneous mycoses. In our series, fungal culture was positive in only 55.7% cases, which was higher than many other studies.[1,4,14,15] Molecular diagnostic techniques such as PCR offer a rapid diagnosis of Fonsecaea sp. and can be used in difficult cases of chromoblastomycosis. However, this facility was not available at our center.

Conclusion

Subcutaneous mycoses are prevalent in northeast India. Chromoblastomycosis and sporotrichosis were two common subcutaneous fungal infections encountered here. There were some cases where a definitive diagnosis of subcutaneous mycoses could not be made. Suppurative granulomas in histopathology might be considered to be of immense utility in diagnosis of such suspicious lesions and empirical itraconazole therapy might be tried.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bordoloi P, Nath R, Borgohain M, Huda MM, Barua S, Dutta D, et al. Subcutaneous mycoses: An aetiological study of 15 cases in a tertiary care hospital at Dibrugarh, Assam, Northeast India. Mycopathologia 2015;179:425-35.
2. Kim MS, Lee SM, Sung HS, Won CH, Chang S, Lee MW, et al. Clinical analysis of deep cutaneous mycoses: A 12-year experience at a single institution. Mycoses 2012;55:501-6.
3. Silva JP, de Souza W, Rozental S. Chromoblastomycosis: A retrospective study of 325 cases on Amazonic region (Brazil). Mycopathologia 1998;143:171-5.
4. Bhat RM, Monteiro RC, Bala N, Dandakeri S, Martis J, Kamath GH, et al. Subcutaneous mycoses in coastal Karnataka in south India. Int J Dermatol 2016;55:70-8.
5. Kimura M, Goto A, Furuta T, Satou T, Hashimoto S, Nishimura K, et al. Multifocal subcutaneous phaeohyphomycosis caused by Phialophora verrucosa. Arch Pathol Lab Med 2003;127:91-3.
6. Hay RJ, Ashbee HR. Fungal Infections. In: Griffiths CEM, Barker J, Bleiker T, Chalmers R, Creamer D, editors. Rook’s Textbook of Dermatology. 9th ed. Singapore: Wiley Blackwell; 2016. p. 32.77.
7. dos Santos Gon A, Minelli L. Melanoma in a long-standing lesion of chromoblastomycosis. Int J Dermatol 2006;45:1331-3.
8. Jacob M, Mathal R, Prasad PV, Bhaktaviziam A. Chromoblastomycosis with squamous cell carcinoma. Indian J Dermatol Venereol Leprol 1988;54:314-7.
9. Minotto R, Bernardi CD, Mallmann LF, Edelweiss MI, Scoferneker ML. Chromoblastomycosis: A review of 100 cases in the state of Rio Grande do Sul, Brazil. J Am Acad Dermatol...
2001;44:585-92.
10. Esterre P, Pecarrère JL, Raharisolo C, Huerre M. Squamous cell carcinoma arising from chromomycosis. Report of two cases. Ann Pathol 1999;19:516-20.
11. Bonifaz A, Carrasco-Gerard E, Saúl A. Chromoblastomycosis: Clinical and mycologic experience of 51 cases. Mycoses 2001;44:1-7.
12. De Araujo T, Marques AC, Kerdel F. Sporotrichosis. Int J Dermatol 2001;40:737-42.
13. Shi D, Zhang W, Lu G, de Hoog GS, Liang G, Mei H, et al. Chromoblastomycosis due to Fonsecaea monophora misdiagnosed as sporotrichosis and cutaneous tuberculosis in a pulmonary tuberculosis patient. Med Mycol Case Rep 2016;11:57-60.
14. Gonzalez Santiago TM, Pritt B, Gibson LE, Comfere NI. Diagnosis of deep cutaneous fungal infections: Correlation between skin tissue culture and histopathology. J Am Acad Dermatol 2014;71:293-301.
15. Pang KR, Wu JJ, Huang DB, Tyring SK. Subcutaneous fungal infections. Dermatol Ther 2004;17:523-31.
16. d'Avila SC, Pagliari C, Duarte MI. The cell-mediated immune reaction in the cutaneous lesion of chromoblastomycosis and their correlation with different clinical forms of the disease. Mycopathologia 2003;156:51-60.
17. Uribe F, Zuluaga AI, Leon W, Restrepo A. Histopathology of chromoblastomycosis. Mycopathologia 1989;105:1-6.