

Case Report

A case series and review of sporotrichosis in Sikkim

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Abstract

Sporotrichosis caused by the fungus *Sporothrix schenckii* has been widely reported from the northern Himalayan belt and the north eastern region of India. Three autochthonous cases of lymphocutaneous sporotrichosis from east and south districts of Sikkim are reported. Fluid aspirate from the nodulo-ulcerative lesions were sent for cytology and fungal culture. *S. schenckii* was isolated on culture and cytological examination in all three cases showed granulomatous reaction. Thermal dimorphism was demonstrated and animal pathogenicity testing was performed. Saturated solution of potassium iodide was used for treatment and the last case was treated with itraconazole and potassium iodide. Awareness of this disease and an extensive environmental study is required to understand the actual burden of this disease.

Key words: Sikkim; lymphocutaneous sporotrichosis; *Sporothrix schenckii*; potassium iodide; itraconazole

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Introduction

Sporotrichosis, caused by the dimorphic fungus *Sporothrix schenckii*, is worldwide in distribution [1]. It has been regarded mainly as an occupational disease usually caused by minor trauma while handling soil or plant material and very rarely as a zoonotic infection associated with felines [2, 3]. Sporotrichosis has been reported from different parts of India [4-9].

Sikkim is a small Himalayan state situated 27.330N; 88.620E coordinates in the eastern Himalayas spread below the world's third highest mountain, the Kanchendzonga. Climatic conditions range from sub-tropical in the foothills to temperate weather as the altitude increases. The total geographical area of the state is 7,096 km² with 540,493 inhabitants counted in the 2001 census. The annual minimum temperature is 11.3°C and the maximum is 19.8°C. The average humidity of Sikkim is 86% with a range between 79-95% and an annual average rainfall of 3,581 mm.

We here report three autochthonous cases of lymphocutaneous sporotrichosis from the state of Sikkim and review cases from north eastern India.

Case Reports

Case 1

A 43-year-old male, employed in a bottle factory at Melli, South Sikkim, visited the surgical outpatient department of Sir Thotub Namgyal Memorial Government Hospital in Gangtok on 29 January 2009. He had complaints of a multiple infected wound on the left forearm and history of injury with glass on the left hand three months back. The wound did not heal and he noticed multiple nodules erupting during the course of infection on the left forearm. He received a broad spectrum antibiotic and was admitted to the surgical ward for further evaluation and daily dressing of the wound with metronidazole ointment. He had no significant medical history, and he drank a moderate amount of alcohol occasionally. Routine blood examination showed Hb- 12% gm, TLC- 4,800 cells/cu mm, DLC- N 60%, L-40%, random blood sugar- 109.4mg/dl. Wound dressing and oral antibiotic was continued with no signs of improvement; therefore, an aspirate from the wound was initiated and sent for pathological analysis on 6 February 2009. He was lost to therapy as he took discharge on request on 9 February.

Figure 1. Nodulo ulcerative lesions along the lymphatics over the hand and forearm (Case 2)



Figure 2. Nodular lesions over the dorsum of the hand (Case 3)



Case 2

A 51-year-old housewife from Assamlingzey, East Sikkim, came to the dermatology outpatient department on 9 February 2010, with multiple nodules on her left forearm. She gave a history of injury to her left little finger while cutting grass in her garden ten months ago. A nodule developed within days which ulcerated forming scaly ulcers with itching and it gradually spread in a linear fashion along the lymphatics over the left upper extremity (Figure 1). She had consulted a local physician who prescribed broad spectrum antibiotics. Her wound did not heal so she finally came to our hospital. She had no associated disease and did not own any pets or domestic animals.

Case 3

A 28-year-old housewife, a resident of Upper Sicchey, Gangtok, came to the dermatology outpatient department on 27 March 2010 with a three-week history of swelling and multiple ulcers on the dorsum of her right hand (Figure 2). She recollected a history of injury with the edge of a steel glass after which a nodulo-ulcerative lesion developed. She gave no history of tuberculosis, diabetes mellitus, or contact with pets.

Cytological examination

The aspirate was stained with Leishman stain and showed granulomas and multinucleated giant cells admixed with non-specific inflammatory cells. Yeast cells were not observed.

Mycological examination

Gram stain of the fluid aspirates sent to the microbiology laboratory showed Gram-positive, round to elongated budding yeast cells in all three cases. They were cultured on two sets of Sabouraud's dextrose agar with gentamicin 0.02 mg/ml and cycloheximide 0.5 mg/ml; one of each set was incubated at 25°C and 37°C. After five days of incubation, white to grayish colonies with aerial hyphae were observed on SDA at 25°C which eventually turned greenish black on further incubation. A slide culture was set up and a conversion to yeast phase was demonstrated by repeated subcultures on brain heart infusion blood agar with gentamicin 0.02 mg/ml and cycloheximide 0.5 mg/ml and incubated at 37°C. Moist, smooth, creamish colonies were observed after three to six days. Gram staining revealed Gram-positive spherical budding yeast cells. Lactophenol cotton blue (LPCB) mount of the slide culture showed thin, hyaline, septate hyphae with

pyriform conidia arranged in a bouquet-like pattern around erect conidiophores and also directly arising from the hyphae.

Pathogenicity testing

For pathogenicity testing, 1 ml of saline washed with the mycelial stage of the fungal isolate was used to perform animal pathogenicity on outbred Swiss albino mice with an intraperitoneal and foot pad inoculation. After two weeks the mice developed orchitis and examination of pus from the testis revealed oval to cigar shaped bodies, and culture recovered *S.schenckii*.

Treatment and outcome

Patients 1 and 2 were started on five drops of a saturated potassium iodide solution three times daily to be increased by five drops gradually until 30 drops three times daily was reached. Patient 1 took discharge on request and was lost to therapy. Patient 2 showed dramatic improvement after six weeks of treatment with complete healing of the ulcers. Patient 3 was initially treated with 200 mg of itraconazole twice daily for seven days with a gap of 21 days for three months. After two months, the patient could not afford itraconazole so she was started on five drops of saturated solution of potassium iodide to be taken three times daily and gradually increased by five drops until 30 drops three times daily was reached. Her lesions gradually disappeared with complete clearance after seven weeks of starting potassium iodide. Treatment was continued for another one month after remission of ulcers for patients 2 and 3.

Discussion

The dimorphic fungus *S. schenckii* is found as a mould in decaying vegetation, sphagnum moss, soil, and other environmental niches throughout the world. When these cells gain entry into host cells they transform into budding yeast cells. The mode of entry can be either direct from any type of thorn prick, cut abrasion or blunt injury, or they may be indirect through bites or scratches from cats and armadillos. Sporotrichosis has been documented in association with a number of other animals such as boars, camels, cattle, chimpanzees, dogs, dolphins, donkeys, fowl, foxes, horses, mules, field mice and rats [1]. Each of our three cases recalled a significant history of trauma before the lesions developed. The source of infection may have been the soil or the surrounding vegetation.

The age and sex distribution of the disease varies from region to region depending upon exposure [10].

It is most commonly seen in adults below 30 years of age but children also form a significant number of sporotrichosis patients. They presumably acquire the infection while playing outdoors and helping with chores [1]. In the present study, the patients ranged in age from 28 to 51 years. The cases reported from India have shown a preponderance of women except for the cases reported from Himachal Pradesh (North India) [7], which showed a male dominance. Differences among reports may be due to the varying distribution of sexes in certain occupations in different regions [1]. In the present case series two patients were female and one was male.

The typical clinical presentation of sporotrichosis is chronic cutaneous and subcutaneous infection [1]. Lymphocutaneous presentation is the most common followed by fixed type. Disseminated visceral, osteoarticular and pulmonary forms of the disease are uncommon and are usually seen in patients with underlying risk factors such as alcohol abuse and immunosuppression, particularly in patients with AIDS. In India, three endemic northeastern states have reported lymphocutaneous infection as the most common presentation, followed by the fixed type in varying proportions (Table 1). The present study reports only the lymphocutaneous type of presentation.

All three patients had no history of travel outside the state and did not own pets or domestic animals; thus the chance of acquiring the disease from the neighbouring endemic state of West Bengal or from a zoonotic source was low. These patients gave obvious histories of injury which made them vulnerable to the infectious propagules of *S. schenckii*.

The recommended drug of choice for cutaneous and subcutaneous sporotrichosis is itraconazole 100 mg orally twice daily for three to six months [11]. Some studies have tried itraconazole as a pulse regimen, *i.e.*, 200 mg twice daily for one week repeated after 21 days for three months, and have suggested it to be an effective and safe alternative [12]. The third patient in our study was started with this regimen but could not afford the drug so the treatment was changed to potassium iodide. Due to financial constraints, a saturated solution of potassium iodide will remain the first drug of choice in low- and middle-income countries such as India. It has various adverse effects such as a metallic taste, enterogastric disorders, hyperthyroidism, salivary gland swelling, rash, and fever. In our present case series, however, none of the patients complained of any adverse effects.

Lesions of sporotrichosis resemble cutaneous leishmaniasis, tuberculosis, chromoblastomycosis, blastomycosis and even chronic staphylococcal skin lesions. A high index of clinical suspicion is required for correct diagnosis of sporotrichosis, and a complete laboratory workup is essential for confirmation of the diagnosis. For example, in our first case, the patient was admitted to the surgical ward after being examined in the surgical outpatient department for more than a week and treated for an infected wound; however, a sample for cytopathological and mycological investigation was sent only after a week of hospital stay. The lesions were misdiagnosed as bacterial infections and broad spectrum antibiotics were prescribed without any further investigation. Lack of awareness of sporotrichosis leads to

Table 1. Clinical profile of patients from the three endemic northeastern states of India

State	Number of cases	Type	Mode of Infection	Region affected	Treatment	Gender
West Bengal [4,14-17]	100	Lymphocutaneous- 66.7% Fixed- 33.3%	Trauma- 22.2% Zoonotic- 11%	Upper limb- 66.7% Lower limb- 33.3%	Saturated solution Potassium Iodide- 88.9% Itraconazole- 11%	Female- 55.6% Male- 44.4%
Assam [4, 13]	45	Lymphocutaneous – 97.3% Fixed-2.7%	Trauma- 89.2%	Upper limb- 48.6% Lower limb- 51.3%	Saturated solution Potassium Iodide- 100%	Female- 62.2% Male- 37.8%
Manipur [5,6]	73	Lymphocutaneous- 63.1% Fixed-36.9%	Trauma- 39.7%.	Upper limb- 53.4% Lower limb- 23.2% Face- 16.4% Other – 6.8%	Saturated solution Potassium iodide- 100%	Female- 33.4% Male- 46.5%

misdiagnosis and subsequent inadvertent use of antibiotics. Treatment of a patient is incomplete unless an appropriate sample is sent to the laboratory for investigation, and this fact must be stressed to health-care workers.

Sikkim's neighbouring states of Assam [13], Manipur [5,6] and West Bengal [4,14-17] have reported a large number of cases of sporotrichosis and sporadic cases are reported from Tripura and Meghalaya [4]. One case of lymphocutaneous sporotrichosis has been reported from Nagaland; the patient was diagnosed and treated in the Christian Medical College and Hospital, Vellore, India [18]. The case profiles of the patients from the three endemic states in northeast India are shown in Table 1. These states in the foothills of the Himalayas share a temperate climate, with heavy rainfall and high humidity. To our knowledge, only one case has been reported so far from Sikkim and this patient was investigated and treated in the neighbouring state of West Bengal [14]. It has been suggested that a humid and moist climate favours the growth of *S. schenckii* [19]. Thus Sikkim remains a lesser known niche for sporotrichosis and the present cases document the autochthonous emergence of sporotrichosis in this geographical region.

As with most fungal infections, diagnosis of sporotrichosis by culture is time-consuming. Serological diagnostic methods give faster results and are easy to interpret. Among those that have been tried and tested are the latex agglutination test (sensitivity 94%, specificity 100%), the tube agglutination test (sensitivity 96%, specificity 98%), and indirect fluorescent antibody detection (sensitivity 90%, specificity 89%) [20]. Detection of antibody by ELISA using mycelial phase exoantigens [21], cell wall antigens [22] and yeast phase antigens [23] have shown sensitivities of 97%, 90% and 100%, respectively. However, ELISA is plagued with cross-reactions with *Exophiala wernickii*, *Fonsecaea pedrosoi*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes* due to sharing of the glycosylated epitopes present in fungus [24]. The Western blot technique using antigen components from 22-70 kDa is able to detect antibodies to major proteins at 40-70 kDa in patients with sporotrichosis [23] and also differentiate between cutaneous and extracutaneous forms by showing darker bands for extracutaneous sporotrichosis, as compared to the cutaneous type [23]. Molecular methods such as nested polymerase chain reaction [25] and restriction fragment length

polymorphism (RFLP) analysis [26,27] are no doubt very sensitive and instrumental in diagnosis of disease when the organism is in a very small amount such as cerebrospinal fluid (CSF). But due to their high costs and the skilled expertise required to perform them, they will remain primarily research tools for epidemiological investigations. In developing countries, routine microscopic and pathological examinations coupled with isolation of the organism in culture will remain the gold standard for diagnosing.

Conclusion

The cases reported here document the autochthonous existence of sporotrichosis and the disease burden in the geographical province of Sikkim. We reiterate the need for a high index of clinical suspicion and the pivotal role of laboratory findings to aid in prompt diagnosis and patient management. Awareness of this disease and an extensive environmental study is required to understand the actual burden of this disease.

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