

Original Article

Cutaneous protothecosis in Hong Kong

香港的皮膚原藻病

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Objective: This article reports the clinical, pathological and microbiological aspects of cutaneous protothecosis in the local scenario. **Methodology:** This is a retrospective study carried out in Social Hygiene Service, Centre for Health Protection, Department of Health, Hong Kong from 1970 to end of 2006. Case records and histopathology reports of patients with the diagnosis of protothecosis were reviewed. **Results:** Four cases of cutaneous protothecosis were identified. They presented variably as papules, plaques, nodules or ulcers over face, trunk or limbs. One patient worked as farmer and hence had potential occupational exposure. Only one patient was "immunocompromised", with a background of newly diagnosed diabetes mellitus and use of topical steroid. The other three patients were immunocompetent. Three cases responded to oral itraconazole treatment with or without surgical debridement. **Conclusion:** Cutaneous protothecosis is a rare infection caused by achlorophyllic unicellular algae *Prototheca wickerhamii* that can occur in immunocompromised as well as immunocompetent hosts. Oral itraconazole is the effective treatment of cutaneous protothecosis.

目的：本文探討本地皮膚原藻病在臨牀、病理學及微生物學上的表現。方法：此項回顧研究於香港衛生署衛生防護中心屬下社會衛生科進行，對1970年至2006年底之間的皮膚原藻病患者的病歷和組織學報告進行回顧。結果：共發現四例皮膚原藻病。分別表現為面部，軀幹和四肢的丘疹，斑塊，結節和潰瘍。一名患者為農民，故可能為職業性影響。僅有一名為免疫障礙患者，為新診斷的糖尿病患者，曾應用局部皮質激素。其餘三人免疫力正常。口服伊曲康唑有效治療三名患者，部分患者經外科清除術處理。結論：皮膚原藻病是一種由無葉綠素單細胞藻類，即小型無綠藻引起的罕見感染，可見於免疫障礙或免疫功能正常的宿主。口服伊曲康唑能有效治療皮膚原藻病。

Keywords: Itraconazole, *Prototheca wickerhamii*, Protothecosis

關鍵詞：伊曲康唑，小型無綠藻，無綠藻

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Introduction

Cutaneous protothecosis is a cutaneous infection caused by achlorophyllic unicellular algae of the genus *Prototheca* described for the first time in 1894 by Wilhem Kruger.¹ It is a rare human cutaneous infection that often affects immunocompromised and sometimes immunocompetent hosts. Skin lesions consist of erythematous patches and plaques which mimic dermatitis or superficial fungal infection. The local epidemiology of the infection is not known. We performed a retrospective survey on cutaneous protothecosis occurring in Social Hygiene Service, Centre for Health Protection, Hong Kong. The clinical features, microbiology, treatment and progress of these cases are presented.

Patients and method

The case records and histopathology reports with a diagnosis of protothecosis were retrieved from 1970 to end of 2006, a 36-year-period. A diagnosis of cutaneous protothecosis was considered definite when organisms of specific morphology were detected in the skin biopsy of lesions. The typical morphology was the presence of spherical organisms of 3-4 microns with internal septation and endospore formation, which can be highlighted by Grocott methenamine stain. Isolation of the pathogen by culture allowed identification of the species but was not mandatory for a diagnosis.

Results

A total of four cases were found in Social Hygiene Service, Department of Health, Hong Kong from 1970 to 2006. Two cases have been reported previously.^{2,3} The diagnosis of cutaneous protothecosis was based on finding the organism in skin biopsy of lesion or isolation of the organism in tissue culture. The clinical and laboratory findings are summarised in Table 1.

Patient 1²

A 29-year-old Chinese female presented in 1970 with a 13-year history of erythematous papules and plaques on the right cheek and temple. There was occasional itching and swelling of the lesions. The lesions progressively increased in size. She reported good past health and there was no preceding trauma to the lesional area. Physical examination showed a large, infiltrated erythematous plaque in right cheek and temple area with yellowish miliary nodules seen under diascopy. The border of lesions was irregular but well-demarcated. She had received courses of antibiotics, anti-tuberculosis drugs, polymyxin B injection and superficial X-irradiation, but no significant improvement was noted. Blood tests including complete blood count, liver and renal function test, plasma protein and immuno-electrophoresis were all normal. Sputum culture for mycobacterium and fungus were all negative. Chest X-ray showed no active lung disease.

Skin biopsy of the right face plaque showed granulomatous inflammation with organisms in the epidermis and dermis. The organisms in intra-epidermal location were small spherical eosinophilic bodies of 3-4 microns with internally small spherical structures. In the dermis, there were larger spherical bodies with an intensely basophilic, internal septate structure. These were endospores in a rosette configuration within sporangia. Culture of the tissue in glucose Sabouraud agar yielded *Prototheca wickerhamii*, a yeast-like creamy white colour colony at first which acquired a dirty yellowish colour upon prolonged incubation. Sputum culture was negative for *Prototheca*.

She was given oral, topical and intralesional injection of amphotericin B respectively. There was no significant response and the plaque progressively increased in size. Repeat biopsy after courses of treatment showed persistence of the infection.

Table 1. The clinical and laboratory findings of four patients with cutaneous protothecosis

	Patient 1 ²	Patient 2 ³	Patient 3	Patient 4
Sex/age	F/29	M/70	M/75	F/79
Occupation	Typist	Farmer	Retired construction site worker	Housewife
Lesion	Right face/temple papules and plaque	Left lower limb ulcerated plaque, right thigh ulcer	Right upper limb, left upper chest, abdomen erythematous nodules	Left arm cellulitis, left ankle ulcer
Time of onset before presentation	13 years	12 months	8 months	3 months
History of trauma	Nil	Nil	Nil	Nil
Immunosuppression	Nil	Nil	Diabetes mellitus, topical steroid	Nil
Differential diagnosis at time of presentation	Cutaneous mycobacterial or deep fungal infection	Cutaneous mycobacterial or deep fungal infection	Deep fungal infection or eczema	Cellulitis
Histopathology	Granulomatous inflammation, endospores and sporangia present	Granulomatous inflammation, endospores and sporangia present	Granulomatous inflammation, endospores and sporangia present	Granulomatous inflammation, endospores and sporangia present
Tissue culture	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>	<i>Prototheca wickerhamii</i>	Negative
Blood culture	Not done	Negative	Not done	Negative
Treatment	Oral Amphotericin B x 2 months, topical Amphotericin B x 2 months, intralesional Amphotericin B x 11 treatments	Oral Itraconazole 200 mg daily for 3 weeks, surgical debridement	Oral Itraconazole 200 mg daily for 4 months initially, for 5 months later for relapse	Oral Itraconazole 200 mg daily for 7 months
Outcome	Unresponsive	Responsive	Responsive	Responsive

Patient 2³

A 70-year-old Chinese male farmer was referred to our Service for management of cellulitis and ulceration on both lower legs for 12 months. There was neither preceding trauma nor recent travel. He was a known hepatitis B carrier. There was no history of diabetes mellitus or immunosuppression. Physical examination showed a large ulcerated erythematous plaque on the left lower thigh extending to the left knee and left upper calf (Figures 1 & 2). Smaller ulcerated lesions were

also seen in right thigh. Culture from an ulcer swab grew *Candida albicans* on the first visit. He was treated with oral itraconazole 200 mg daily for 2 weeks. The lesion almost healed, but recurred after he had defaulted follow-up for 1 month. This time, discrete scaly erythematous papules were noted on face, left forearm, and left leg.

Skin biopsy of the ulcerated lesion on the left leg showed suppurative granulomatous inflammation in dermis and subcutis with spores and sporangia readily demonstrated by Grocott methenamine



Figure 1. The ulcerated lesion in left knee of patient 2.



Figure 2. The erythematous ulcerated plaque in left calf of patient 2.

stain. Lactophenol cotton blue stain of the organism showed sporangia-containing endospores (Figure 3). Culture grew *Prototheca wickerhamii*. He was treated with itraconazole 200 mg daily for 3 weeks and surgical debridement. Although his subsequent antifungal sensitivity test showed resistance to itraconazole and sensitivity to amphotericin B, he responded to the treatment and the lesions resolved.

Patient 3

A 75-year-old Chinese retired construction site worker presented with an erythematous plaque on the right forearm for 8 months. In the past five years prior to the presentation, he was intermittently seen in the dermatological clinic for chronic eczema and had been given topical corticosteroids and oral antihistamines. The right upper limb plaque was initially treated as eczema

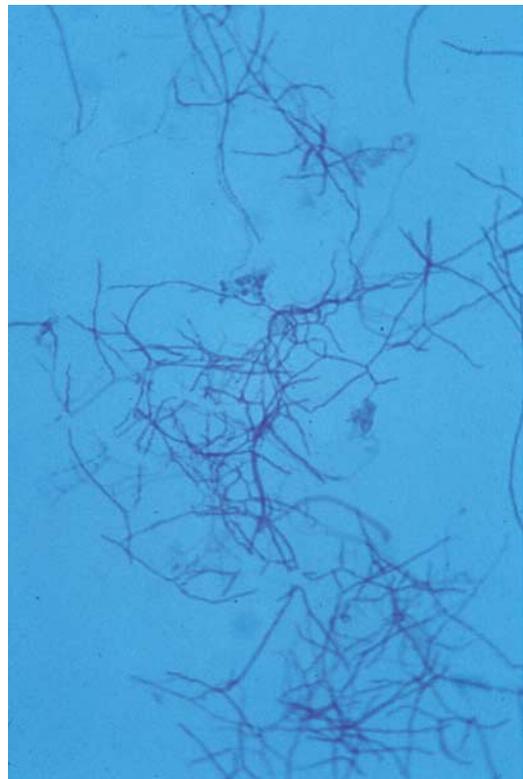


Figure 3. Lactophenol cotton blue stain showing *Prototheca wickerhamii* with sporangia containing endospores (x 200).

with no improvement. The lesion was itchy but non-painful. There was no preceding trauma to the area. He did not keep any fish, pets and no gardening activities were reported. Physical examination showed extensive plaques and nodules on the right forearm, elbow and upper arm (Figure 4). Nodules were also noted on left upper chest (Figure 5) and left abdominal area.

Skin biopsy of a right forearm plaque revealed similar histopathology as the above two cases (Figures 6 & 7). Culture of the biopsy tissue yielded

Prototheca wickerhamii. Blood test results for complete blood picture, liver and renal function tests were normal. However, his spot glucose was 9.5 mmol/l.

The patient was treated with itraconazole 200 mg daily for four months. The skin lesion resolved and repeated biopsy and culture four months after treatment revealed no organisms. Further evaluation of his elevated blood glucose confirmed a diagnosis of diabetes mellitus and he was



Figure 4. Erythematous nodules and plaque in right upper limb in patient 3.



Figure 5. Extensive erythematous nodules in left upper chest of patient 3.

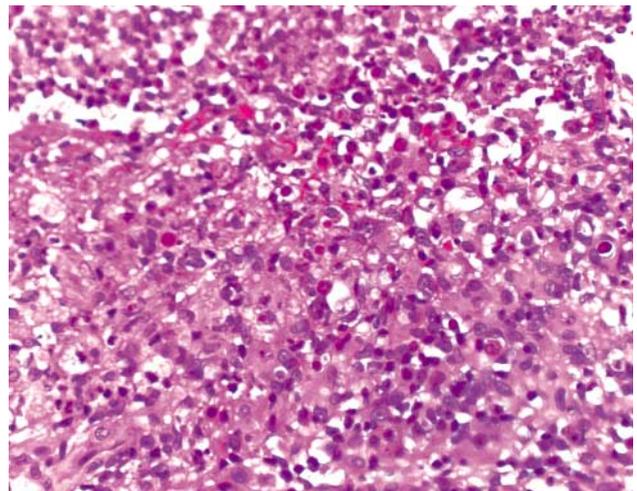


Figure 6. Suppurative granulomatous inflammation with admixed spores demonstrated in skin biopsy from patient 3 (H&E stain, x 400).

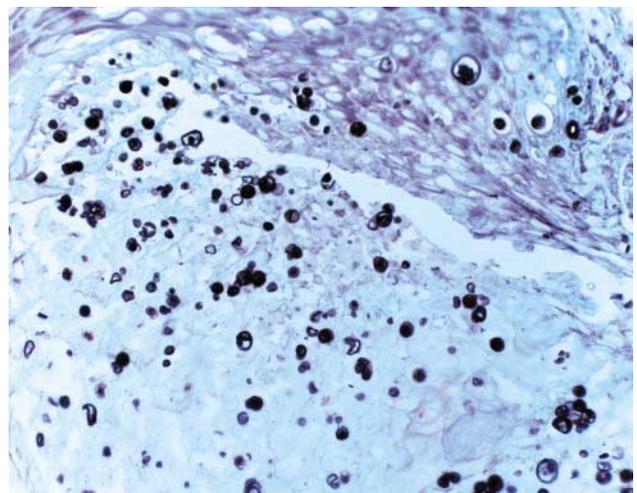


Figure 7. Spores and morula form sporangia demonstrated in skin biopsy from patient 3 (Grocott stain, x 400).

advised on dietary restriction as an initial management. After eight months, he had a relapse of the skin lesion in right forearm, right shoulder and left forearm. A repeat skin biopsy confirmed a relapse of cutaneous protothecosis though culture this time was negative for the algae. He was treated as clinical relapse with itraconazole 200 mg daily for five months. The lesions completely resolved. Repeat skin biopsy and culture two months after stopping treatment showed no organisms.

Patient 4

A 79-year-old Chinese housewife presented with a 3-month history of left upper limb and left ankle erythematous plaque and ulcer. There was no history of preceding trauma. She had good past health except for a history of psoriasis which was then in remission. She gave no history of diabetes mellitus and immunosuppression. Physical examination showed extensive erythematous plaque with ulcerations in the left arm and forearm (Figure 8). There was a small superficial ulcer on the left ankle with a clean base. She was treated as cellulitis with intravenous ampicillin and cloxacillin as an in-patient, but the skin lesions persisted.

Skin biopsy of the left forearm lesion for histopathology showed typical features of cutaneous protothecosis. However, culture of biopsy tissue did not demonstrate any *Prototheca* species. She was treated with



Figure 8. Extensive erythematous plaque with ulcerations in left arm and forearm of patient 4.

itraconazole 200 mg daily for 7 months. The lesion completely resolved, leaving post-inflammatory hyperpigmentation.

Discussion

Epidemiology

Prototheca species are ubiquitous, aerobic achlorophyllic unicellular algae requiring an exogenous source of carbon and nitrogen as nutrition. They are adapted to both artificial and natural environments. *Prototheca* had been isolated from various aquatic sources such as lakes, streams, sewage, industrial sludge ponds and marine water. They can also be isolated from mucous flux of trees, infected potato skin, soil and animal wastes.^{4,5}

This organism is of low virulence to the immunocompetent host. However, a case study by Iacoviello et al found that at least 50% of patients were immunocompromised.⁶ In another review by Leimann et al, near 50% of patients were immunocompromised to some extent.⁷ The immunocompromised state was secondary to steroid use, organ transplantation, diabetes mellitus, haematological or solid organ malignancy. In a small report on five patients, all were immunocompromised, mostly due to systemic or topical steroids.⁸ However, a considerable number of patients such as those in our review (patient 1, 2 and 4) were apparently healthy and immunocompetent. Only patient 3 had a diagnosis of diabetes mellitus after the presentation and history of intermittent use of topical steroids.

Protothecosis can present clinically in three forms: cutaneous lesions, olecranon bursitis and systemic protothecosis. Most cases present as localised cutaneous lesions.^{3,7,8} The infection usually spreads locally and insidiously. In a review of 107 patients by Leimann et al,⁷ protothecosis (both cutaneous and non-cutaneous) most frequently occurred in

the upper extremities in 51 cases (including 18 cases of olecranon bursitis), followed by 20 cases occurred in lower extremities, 10 cases on the scalp and face, 8 cases on the trunk and 18 cases of systemic infection involving the intestines, peritoneum, lymph nodes, liver, gall bladder, meninges, nasopharynx and heart. In four patients, the infection was considered disseminated. Farming is an occupation associated with a high susceptibility to the infection.⁸ Only patient 3 was a farmer and occupationally exposed to the infection. There was no obvious source of exposure in the other three patients and there was no sex or age predilection although most patients were over 30 years of age. All our patients were adults on presentation. There have been 10 cases described in children of less than 18 years of age.⁷

In cutaneous protothecosis, the lesions are often located in exposed areas like the extremities and face.⁷ The lesions have a variable appearance: erythematous plaques, nodules, verrucous lesions, vesicles, pustules and ulcers.^{2,3,7,8} The organism was introduced into the skin by local trauma or percutaneous inoculation, usually trivial in nature. The incubation period is in terms of weeks to months. In all of our patients, clinical presentation was typical with erythematous plaques, nodules and ulcers in the extremities (patient 2, 3, 4) and face (patient 1).

Cutaneous protothecosis usually runs an indolent course. The lesions usually remain localised. Dissemination of cutaneous disease is possible in patients with local or systemic immunosuppression.

Diagnosis

Very often, the diagnosis is not suspected initially but the unusual clinical course should raise the suspicion of unusual or deep fungal infection and skin biopsy is therefore required for further evaluation.

Histologically, cutaneous protothecosis is characterised by suppurative granulomatous

infiltration in the dermis and subcutaneous tissue. The detection of thick-walled sporangia with internal septation and sporangiospore formation are diagnostic of protothecosis. The characteristic morula-like structures are easily distinguished from other deep fungal infections with spore-like structures such as cryptococcosis, blastomycosis, rhinosporidiosis and coccidioidomycosis.^{3,5} All four of our patients had skin biopsy done which showed typical suppurative granulomatous inflammation in the dermis and demonstration of typical endospores and sporangia of *Prototheca*. Identification of *Prototheca* species requires culture confirmation. *Prototheca* forms creamy yeast-like colonies on Sabouraud's medium when grown at an environmental temperature between 25 to 32°C. A lactophenol cotton blue stain should be performed in every culture to reveal typical sporangia-containing endospores. It can prevent misidentification with the utilisation of API 20C.⁹ PAS, Gridley and Gomori-Grocott stains were superior to H&E for detecting *Prototheca* cells as the cell wall and internal sporangiospores of intact cells are well delineated.¹⁰ *Prototheca* consists of three species: *P. wickerhamii*, *P. zopfii* and *P. stagnora*. The first two species are pathogenic in human with the majority of cases caused by *P. wickerhamii*. The species can be differentiated by use of 50 µg clotrimazole discs.¹¹ Three of our patients (patient 1, 2, 3) had a positive tissue culture of *Prototheca wickerhamii*.

Management

Cutaneous protothecosis can be treated by surgical excision if the lesions are localised.¹² Treatment is more effective when surgery is combined with pharmacological therapy. Antifungal agents such as amphotericin B,^{8,13} itraconazole,^{3,14} fluconazole^{8,15} and ketoconazole^{8,16} are reported to be useful. However there has been no consensus in treatment owing to the small number of patients and variable response seen with different agents. Combination of amphotericin B and tetracycline has been used with success by some authors.^{8,17} Local application of amphotericin B has been

reported to be effective,^{6,8} but this was not achieved in patient 1. In our review, patient 2, 3 and 4 were responsive to treatment with oral itraconazole 200 mg daily. Patient 2 had surgical debridement in addition to itraconazole therapy. Itraconazole was used as it was less hepatotoxic than ketoconazole. Amphotericin B is notorious for its nephrotoxicity and it has to be given by intravenous infusion. Therefore it is reserved for serious or resistant infections. New antifungal agents such as voriconazole has been used with success in a case report recently.¹⁸

Patient 3 gave a complete response to four months of itraconazole treatment. However, he had a clinical and histological relapse eight months after stopping the itraconazole. Resumption of treatment with itraconazole 200 mg daily for five months subsequently was able to achieve a cure for the relapse. The skin biopsy taken after treatment showed histological and microbiological remission. In view of the underlying diabetes mellitus and history of relapse, regular follow up monitoring of this patient is necessary.

Conclusion

Cutaneous protothecosis is a rare cutaneous infection in this locality. It may cause considerable morbidity and even mortality especially in immunocompromised hosts. In the four cases reviewed, three were considered immunocompetent. It is not certain whether some subtle intrinsic immune defect may be present in patients suffering this unusual infection. Clinicians must be aware of this entity when encountering suspicious skin lesions. A skin biopsy should be promptly performed to enable early diagnosis and treatment. Oral itraconazole is recommended as a first line treatment as it is less hepatotoxic, better tolerated, more convenient to administer and cheaper than intravenous amphotericin B. Surgical treatment increased treatment efficacy when combined with systemic antifungal agents.

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