Case Report

Cryptococcal infection in a post renal transplant patient presenting as a cutaneous nodule

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DOI: http://doi.org/10.4038/jdp.v11i2.7697

Submitted on 27.11.2016 Accepted for publication on 18.12.2016

Introduction

Cryptococcus neoformans is an encapsulated fungus capable of causing life threatening opportunistic infections in immunocompromised patients. It has a worldwide distribution and is most commonly found in bird droppings and contaminated soil.

Risk factors for acquiring cryptococcosis include HIV infection, solid organ transplantation (SOT) and other forms of innate and acquired immunosuppression (1). Infection is usually acquired by inhalation of dehydrated yeasts or basidiospores leading to the establishment of a primary pulmonary infection (2). Subsequent to initial infection or reactivation, there can be haematogenous dissemination preferentially to the central nervous system (CNS) and a variety of other organs like skin, soft tissue and bone, depending on the immune status of the host (3). Immunocompetent patients can be asymptomatic or have limited pulmonary disease, while immunocompromised patients tend to have disseminated disease with frequent CNS involvement and a fatal outcome.

Notably in the past decade or so, there has been a shift in the type, presentation and incidence of opportunistic infections worldwide. This has been partly due to the expansion of the 'at-risk population' as a result of both newer therapeutic modalities such as transplantation and immunosuppression coupled with advances in life support, enabling longer survival of debilitated patients. In Sri Lanka too, where organ transplantation is becoming commoner, it is imperative that physicians, radiologists, microbiologists and pathologists become aware of these changing patterns ofdisease.

The first case of cryptococcosis diagnosed and confirmed in Sri Lanka was reported by Jayewardena et al. in 1963 in the central nervous system (4). Thereafter published reports of cryptococcosis are sparse and limited to isolated case reports. However, recent estimates extrapolated from unpublished data from the Medical Research Institute indicate an estimated annual national case-load of 13 cases (5).

We describe a case of cryprococcosis with central nervous system and skin involvement, in a patient on immunosuppression following a kidney transplant five years prior.

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Case report

Clinical history

A 63-year-old man who had undergone renal transplant 5 years back, presented with nausea and vomiting of 3 days duration. He had headache, hearing impairment, vertigo and a cough for three weeks. He was on

treatment for diabetes, hypertension and ischaemic heart disease in addition to his immunosuppressant medications.

On general examination, he was found to have an ulcerated skin nodule of 2 cm with everted edges on the forehead, which had been enlarging slowly for the past 3 months. Examination of the respiratory system revealed bilateral crepitations; other clinical details were not available.

Pathological features

Excision biopsy and microscopy of the forehead nodule revealed ulcerated skin with areas of granulomatous inflammation containing scattered histiocytes, lymphocytes and foreign body type giant cells. Multiple dermal neutrophil micro-abscesses containing numerous organisms were noted (Fig 1). The organisms were encapsulated, spherical to oval yeast forms (5-10µm) with a clear halo around them and displayed narrow-based budding. Clustering of organisms within macrophages, as seen in histoplasmosis, was not evident. Necrotising vasculitis which often occurs in histoplasmosis and candidiasis was also absent. The organisms stained magenta colour with Periodic acid-Schiff (PAS), black with Grocotts methenamine silver (GMS) and blue with the Alcian blue stain. The Masson Fontana stain revealed a positive argentaffin reaction (Fig 2). A presumptive diagnosis of cutaneous Cryptococcosis was made on the basis of this morphological appearance. Exclusion of cryptococcosis in other organs was suggested as isolated skin infection is known to be highly unusual.

Cryptococcus neoformans antigen was found to be positive in the CSF confirming central nervous system involvement in this patient. Cryptoccal antigen in the CSF detects capsular antigens and shows sensitivity and specificity of over 90% (6). India ink stain however, was not able to identify organisms in the CSF and cultures were also negative. Although the patient was found to have a low haemoglobin, the white cell counts were normal. The serum creatinine levels were elevated. Chest X-ray revealed bilateral interstitial shadows. High resolution computed tomography scan (CECT) of chest showed bilateral reticulonodular shadows in the lungs. Tissue biopsy of lung lesions was not performed.

The patient was diagnosed as having cryptococcosis of the central nervous system and skin with possible lung involvement. He was treated initially with intravenous Amphotericin B for which he developed cardiotoxicity; hence, changed to oral fluconazole. However, the patient unfortunately succumbed to the infection two weeks later.

Discussion

Immunocompromised patients including SOT recipients are susceptible to opportunistic fungal infections. After Candida and Aspergillus, Cryptococcal infection (which is classically associated with HIV infection) is the third common fungal infection reported among SOT patients (3). The majority of patients present later (median of 20 months post transplant) rather than immediate post-transplant, with male sex being a predisposing factor. This is often linked to higher exposure to environmental risk factors such as bird droppings, decaying wood and soil (3). Our patient was male and presented five years after renal transplant.

Humans inhale cryptococcal yeasts resulting in the lung often being the primary site of infection. From there, the organisms can disseminate to the central nervous system, skin, bone or other tissues. Involvement of more than one organ is commoner in organ transplant patients. CNS involvement in the form of meningitis, meningoencephalitis and cryptococcomas are well known. However, both pulmonary and central nervous system symptoms can be subclinical in a significant proportion of patients. As primary cutaneous cryptococcosis is rare, cutaneous lesions in SOT patients are often a cue to look for more extensive disease elsewhere (7).

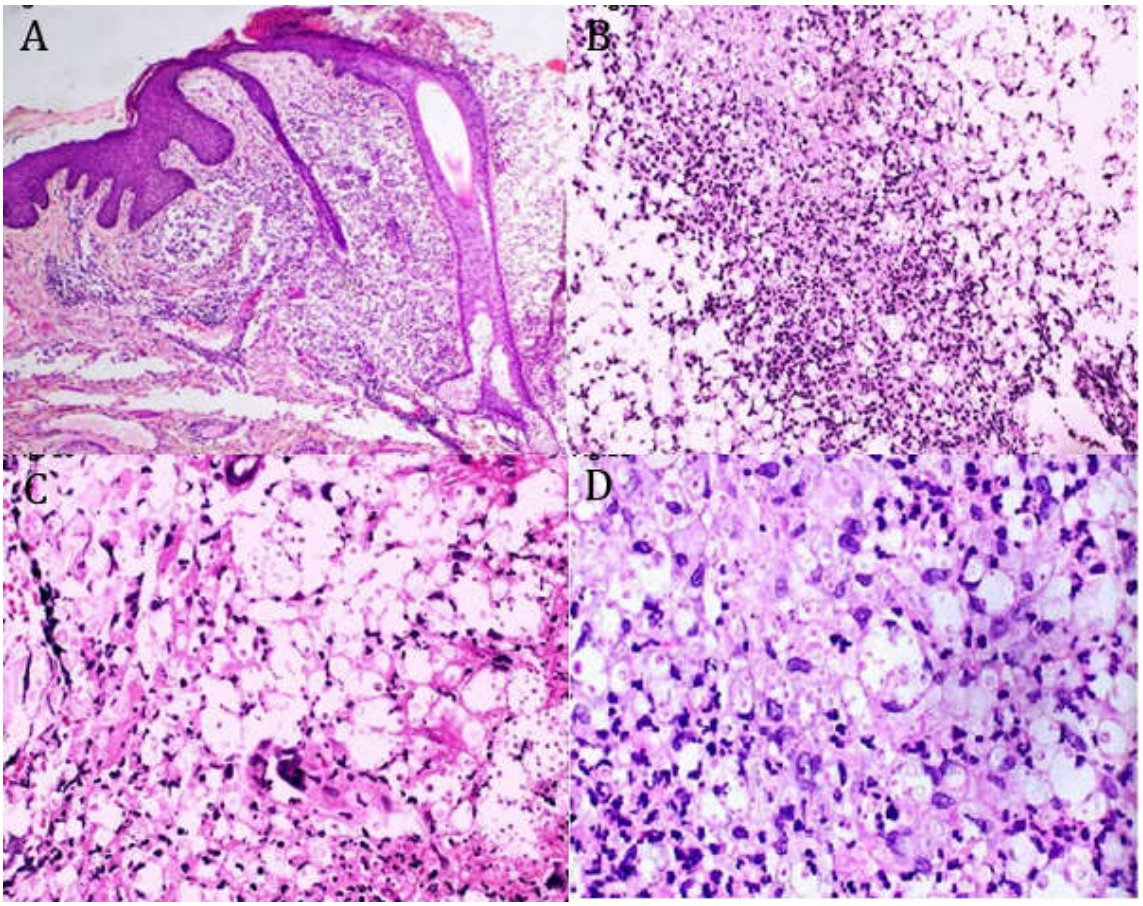

Figure 1.Histology of excision biopsy of the cutaneous nodule.A) Ulcerated skin tissue with heavy dermal inflammation (H&E x40); B) Areas of suppuration with neutrophil micro-abscesses containing numerous yeast-form organisms (H&E x10); C) Numerous organisms with a chronic inflammatory infiltrate comprising histiocytes, lymphocytes and foreign-body type giant cells (H&E x20); D) Numerous "soap bubble" like intracellular and extracellular organisms (H&E x40).

This patient is likely to have had cryptococcosis involving the CNS and skin on presentation as evidenced bycryptococcal antigen positivity in the CSF combined with the presence of a fungal infection morphologically consistent with cryptococcosis in the skin. It is likely that the CNS infection was subclinical and subsequently worsened. The skin lesion of three months was not regarded as important by the patient. These factors are likely to have contributed to the late diagnosis and poor outcome of the disease in this patient. It is vital to recognize that cutaneous lesions which occur in patients after transplantation could have an infectious or neoplastic aetiology. These should be actively looked for and excluded becauseof their potential to cause life-threatening complications if not recognized and managed early.

The laboratory diagnosis of cryptococcosis includes conventional methods such as direct visualization by the India ink method, histopathology, culture and antigen testing.Newer methods include immunohistochemitry, in-situ hybridization and PCR. The tissue reaction pattern is

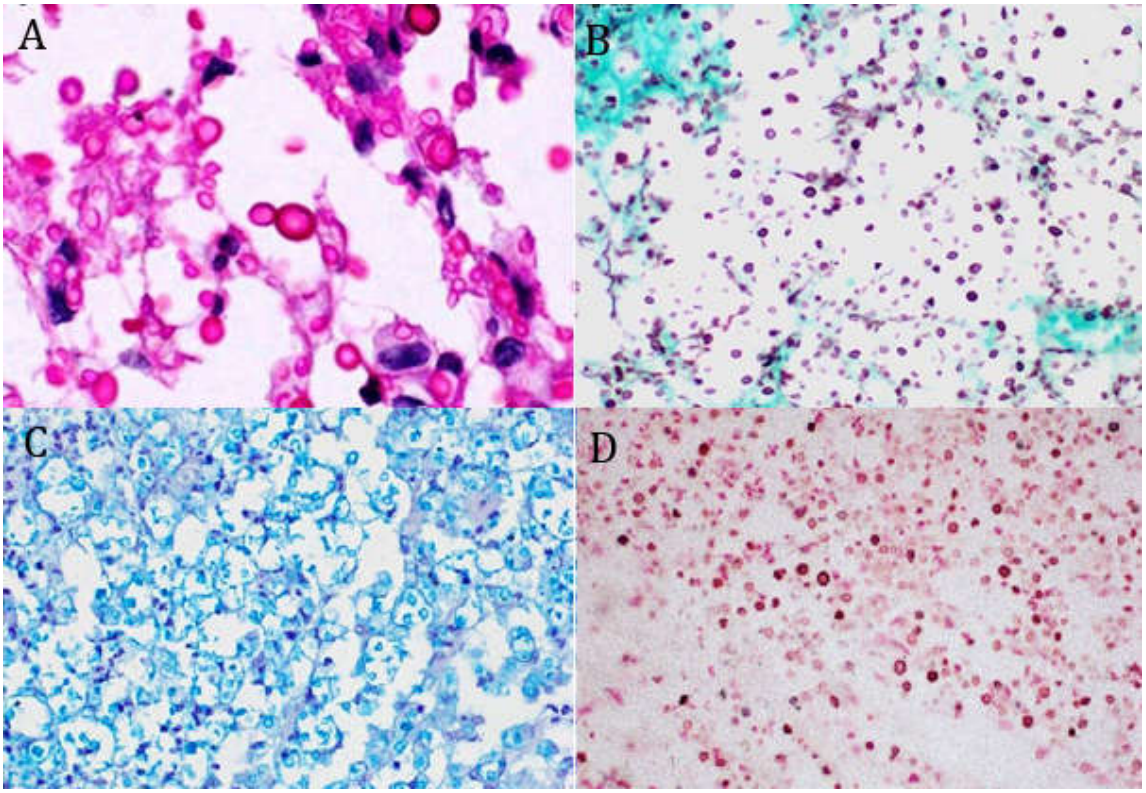

Figure 2.Special stains performed to identify the fungus in the excision biopsy of the cutaneous nodule.A) Intense fuchsia pink staining of cryptococci showing narrow based budding (PAS x40 closeup); B) Yeast-form organisms staining positive with Grocott methenamine silver stain (GMS x40); C) The thick polysaccharide capsule of the organisms stains with Alcian blue (Alcian blue x40); D) Argentaffin reaction seen in the cell wall of Cryptococcus due to the presence of melanin (Masson Fontana x40).

dependent on host immune status and can range from minimal reaction with numerous extracellular organisms to varying degrees ofacute and chronic inflammation including suppuration, necrosis, well formed granuloma with fibrosis and rarely a plump spindle cell proliferation admixed with lymphocytes and plasma cells imparting an inflammatory pseudotumour-like appearance with lymphocytes and plasma cells (8). On haematoxylin and eosin stains, the organismsare round to oval encapsulated yeasts surrounded by a clear halo. Their wall stains with PAS and GMS.The thick polysaccharide capsule of the Cryptococcus stains positive with Alcian blueand mucicarmine. Variability in the amount of the polysaccharide capsule can diminish the clear halo on H&E and abolish the capsular staining seen. Positivity for the Masson Fontana stain is recognised to distinguish cryptococcosis from similar sized fungi like *Candida spp.* and *Histoplasma* as the Cryptococcus produces melanin, giving a positive argentaffin reaction (6).

India ink stain has been used historically as a negative stain to identify Cryptococcus in CSF. Fungal culture confirms ongoing infection and is vital for antifungal sensitivity testing when indicated. Crypococcal antigen testing on serum or CSF using latex agglutination or enzyme immune assay show a sensitivity and specificity of over 90% (6). False positives can

occur with *Trichosporon sp.*, *Klebsiellapneumoniae*, in patients with positive rheumatoid factor or if the reagent was incubated with the specimen beyond the specified time (6). False negatives can occur due to low fungal burden or a prozone phenomenon (6). Tests directed against capsular antigens may also be negative in capsule deficient strains (9).

In the present case the combination of a skin lesion containing yeast forms morphologically consistent with cryptococcus infection which were PAS, GMS, Alcian blue and Masson Fontana positive, in a patient with CNS symptoms and positive CSF cryptococcal antigen were considered highly suggestive of a cryptococcal infection, although there was no microbiological confirmation. However, the lack of specific microbiological demonstration of the organism in vitro was a limitation.

Biopsy of a skin lesion is a quick, cost effective and readily available method of providing a presumptive or definitive diagnosis of cryptococcosis, especially if antigen testing is unavailable. Histopathological evaluation can characterize the host inflammatory response, demonstrate the extent of organism load, confirm tissue invasion and infection by capsule deficient strains where the antigen tests can give false negative results.

Conclusion

Cryptococcosis is an opportunistic fungal infection which can present in routine practice in Sri Lanka especially among transplant recipients on immunosuppression. In this clinical setting, a high index of suspicion for fungal infection is vital, as delays in diagnosis lead to almost certainly devastating outcomes. Though unusual, cryptococcosis in immunocompromised patients can present with cutaneous involvement as respiratory and central nervous system symptoms may initially be mild and subclinical. Biopsy of skin lesions and an active search for systemic involvement will facilitate early diagnosis and optimise management. Histopathological evaluation of tissue is a valuable adjunct to antigen testing and culture and provides a quick, cost-effective and widely-available means of diagnosis.

Acknowledgements

We acknowledge the technical staff of the Department of Pathology, Faculty of Medicine, University of Colombo for the preparation of histology slides.

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