

Células atípicas de *Cryptococcus neoformans* var. *neoformans* observadas em líquido cefalorraquidiano

Atypical *Cryptococcus neoformans* cells isolated from cerebrospinal fluid samples

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RESUMO

Muitos fungos patogênicos apresentam morfologias diferentes nas formas parasíticas e saprofíticas. Essas leveduras podem apresentar morfologias diversas como hifas curtas e aberrantes, formas gigantes em pus fresco, abscesso cerebral, cortes histológicos e presença de cápsula espessa geralmente no sorotipo B em tecidos. No presente relato é descrito o caso de um homem de 39 anos de idade, solteiro e HIV positivo, com os seguintes sintomas clínicos: candidíase oral, tuberculose pulmonar, insuficiência cardíaca, meningite por *Corynebacterium sp* e neurocriptococose por *C. neoformans* var. *neoformans*. O paciente foi tratado com anfotericina B durante 45 dias, com dose total de 1960mg. Foram observadas e isoladas do líquido cefalorraquidiano (LCR) células atípicas de *C. neoformans* de diferentes tamanhos e formas de blastoconídios. O exame direto do LCR em tinta da China apresentou células ovóides, ramificadas, com pseudo-hifas envolvidas por uma fina cápsula. A variedade foi identificada como *C. neoformans* var. *neoformans*. O teste de suscetibilidade aos antifúngicos realizados segundo a técnica de EUCAST indicou que a cepa isolada era sensível a: anfotericina B (Concentração Inibitória Mínima - MIC = 0,12µg/mL), Fluconazol (2µg/mL) e itraconazol (0,06µg/mL). Os relatos anteriores sobre leveduras atípicas descrevem a ocorrência de blastoconídios múltiplos e irregulares em materiais biológicos humanos.

Palavras-chave. células atípicas, *Cryptococcus neoformans*, morfologia.

ABSTRACT

Most pathogenic fungi show dual morphology features in their saprophytic and parasitic stage. Occasionally, yeast cells produce short and aberrant hyphae, or large cells which can be seen on granulomas histological analysis, and otherwise as giant forms in fresh pus from abscesses, or also as highly encapsulated - serotype B in tissue specimens. The present study reports a HIV positive 39 years old single male patient, who presented oral candidiasis, pulmonary tuberculosis, cardiac insufficiency, *Corynebacterium sp* meningitis, and neurocryptococcosis due to *C. neoformans* var. *neoformans*. The patient was treated with amphotericin B for 45 days, who received approximately a total of 1,960 mg of drug. Atypical *C. neoformans* cells in diverse sizes and blastoconidia forms were isolated from cerebrospinal fluid (CSF). CSF stained with a China ink preparation revealed the presence of ovoid, branched, and pseudohyphae forms surrounded by a thick capsule. The isolate variety was identified as *C. neoformans* var. *neoformans*. Antifungal susceptibility testing performed by means of EUCAST⁵ technique indicated that the strain was sensitive to amphotericin B (MIC = 0.12µg/mL), fluconazole (2µg/mL), and itraconazole (0.06 µg/mL). The previous reports on atypical yeast cells form described the occurrence of multiple and irregular blastoconidia in human clinical samples.

Key words. atypical cells, *Cryptococcus neoformans*, morphology.

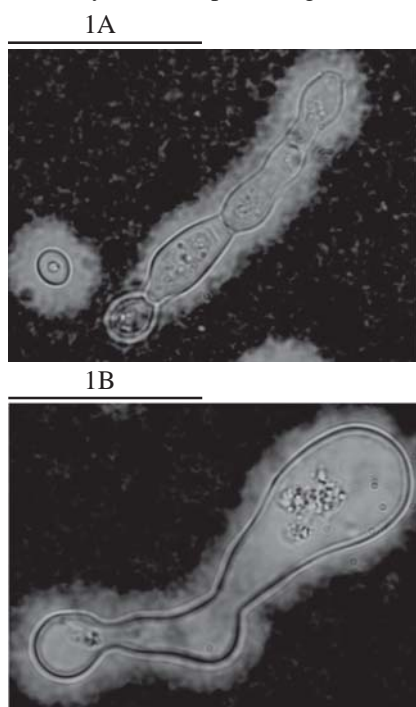
INTRODUCTION

It is well established that pathogenic organism grown in a parasitic state are different in many aspects from those grown on culture media¹. Most pathogenic fungi show dual aspects of the morphology of fungi in their saprophytic and parasitic state². Giant forms of *C. albicans*^{3,4} and *C. neoformans* have occasionally been observed in vivo. A *C. neoformans* yeast cell has an average diameter of 8-10µm. The yeast cell is surrounded by a polysaccharide capsule of various thicknesses. Occasionally, yeast cells have been reported to produce short aberrant hyphae⁵ large cells may be seen histologically in *C. neoformans* granulomas⁸ giant form in fresh pus⁶, large cells isolated from cerebral abscess⁷ and highly encapsulated *C. neoformans* serotype B from tissue^{4,8}.

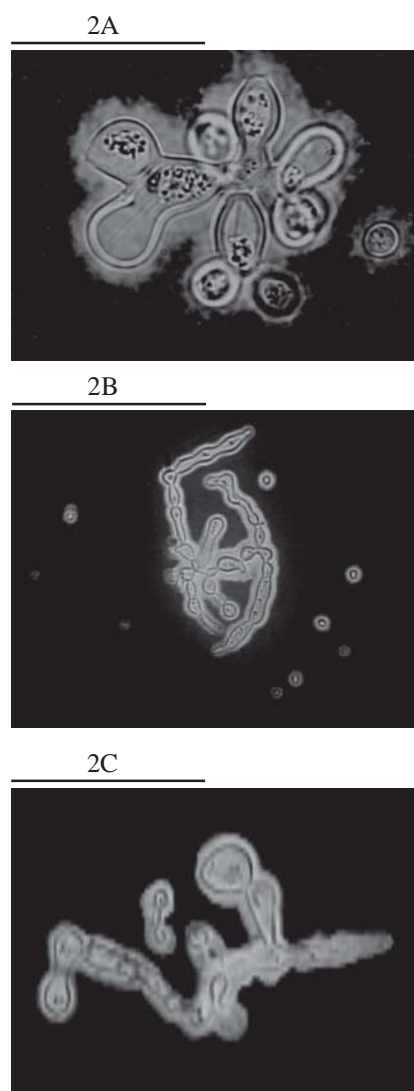
CASE RELATE

This case relate a single man, admitted at the Instituto de Infectologia Emílio Ribas, São Paulo-Brazil, 39 years-old, positive HIV, presented oral candidosis, pulmonary tuberculosis, cardiac insufficiency, *Corinebacterium* meningitis and neurocryptococcosis to *C. neoformans* var. *neoformans*. The patient was treated with amphotericin B for 45 days, total doses 1960mg.

Pleomorphic *C. neoformans* cells were isolated from CSF, and an China ink preparation from Central Nervous System (CNS) revealed different sizes and pseudohyphae forms (Figures 1A and 1B), blastoconidia, ovoid, branched and pseudohyphae forms surrounded by a thick capsule (Figure 2A, 2B and 2C).



Figures 1A and 1B. Pleomorphic *C. neoformans* with different sizes and pseudohyphae forms isolate from CSF of Central Nervous System of a man, positive HIV and ink China preparation.



Figures 2A, 2B and 2C. Pleomorphic *C. neoformans* with blastoconidia, ovoid, branched and pseudohyphae forms isolate from CSF of Central Nervous System of a man, positive HIV and ink China preparation.

The CNS samples were cultured in Niger agar. Brown pigmentation and mucoid colonies were found on all media. The culture samples were also tested by the API 20C AUX (Biomerieux) which revealed an assimilation pattern of *C. neoformans*. Urease activity was observed on the Christensen's urea agar. A subculture of the isolate was tested for varieties on the CGB agar medium⁹. The variety of the isolate was *neoformans*.

The antifungal susceptibility testing performed by EUCAST, Cuenca-Estrella¹⁰ method indicated a sensible strain to amphotericin B (Minimal Inhibitory Concentration: MIC= 0.12µg/mL), fluconazole (2µg/mL) and itraconazole (0.06µg/mL).

DISCUSSION

The origin of atypical *C. neoformans* in our patient is unknown. Sometimes HIV patient present atypical yeast like cells and filaments. The purpose of this paper is to alert the mycologist for the importance of the China ink preparation to demonstrate capsule and showing diversity of morphology of *C. neoformans* presented in immunocompromised patients.

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