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ORIGINAL ARTICLE

PARACOCCIDIOIDOMYCOSIS DUE TO SMALL FORMS OF *Paracoccidioides*. A REPORT OF 12 CASES AND REVIEW OF THE LITERATURE

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ABSTRACT

Paracoccidioidomycosis (PCM) is a systemic mycosis with a geographic distribution limited to Latin America. PCM is caused by species in the genus *Paracoccidioides*, which usually appear in tissues as large yeasts, 5 to 30 μm in size. The daughter cells are attached to the parent cell by a narrow neck. Sometimes smaller forms occur (1 to 4 μm). These can be confused with other fungi, such as *Histoplasma capsulatum* and unencapsulated *Cryptococcus* variants. Twelve cases of PCM were reported with small forms of *Paracoccidioides*. The aim of this paper is to focus on the possibility of differential diagnosis with other systemic mycoses.

KEY WORDS: Paracoccidioidomycosis; *Paracoccidioides*; small forms.

INTRODUCTION

Paracoccidioidomycosis (PCM) is a systemic mycosis with a geographic distribution limited to Latin America (Brummer et al, 1993). PCM is caused by species in the genus *Paracoccidioides*. PCM was previously thought to be caused solely by *P. brasiliensis*, but a new species, *P. lutzii*, was recently discovered in the central-western region of Brazil (Brummer et al, 1993; Gegembauer et al, 2014).

Paracoccidioides species exists in the mycelial form in nature but undertake on the yeast form at body temperature. It usually appears in tissues as large, thin-necked budding yeasts, 5 to 30 μm in size. Multiple buds surround a parent cell resembling a "ship's wheel".

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Yeast cells of *Paracoccidioides* that do not demonstrate multiple budding may, at first, be occasionally confused with other fungi, but after thorough examination and other laboratory diagnostic findings can lead to the correct diagnosis. Small forms of *Paracoccidioides* (1 to 4 μm) have some resemblance to the yeast cells of *H. capsulatum*; larger and more irregular sized forms may be confused with the unencapsulated yeast of the *Cryptococcus* species.

Twelve cases of PCM with small forms of *Paracoccidioides* were reported. The aim of this paper is to investigate the possibility of differential diagnosis with other systemic mycoses.

PATIENTS AND METHODS

The medical records of twelve patients with PCM at the Santa Casa Hospital in Porto Alegre, Rio Grande do Sul, Brazil were reviewed, all of which presented small forms of *Paracoccidioides*. Six of the cases have not been published yet, while the other six have been previously reported (Londero et al, 1980; Santos et al, 1997; Severo et al, 1979; Severo et al, 1980; Severo & Londero, 1981; Severo et al, 1985). This study was conducted with the permission of the Medical Research Ethics Committee of the Santa Casa (Protocol number 461.482/2013).

Direct examination of wet-mount preparations of sputum samples and/or tissue sections stained by hematoxylin and eosin (H&E) and Grocott-Gomori's methenamine silver stain (GMS) allowed for mycological diagnoses which were based on the identification of multi-budding elements of *Paracoccidioides* yeast cells. Fungal cultures could not be performed, except in one case, because the biopsies were stored in formaldehyde. Serologic tests were performed using the agar-gel immunodiffusion (ID) method.

RESULTS

Ten patients had symptoms consistent with PCM. Two were asymptomatic. All cases occurred in adult smokers who resided in an endemic area. Ten patients were male. The two female patients were in menopause. Patient ages ranged from 33 to 68 years of age (mean age = 55,6 years). Symptoms and other characteristics of all patients are summarized in the Table.

Histopathological examination of lung, oropharyngeal and laryngeal biopsies showed the presence of granulomas in nine patients, mostly with central necrosis. Small yeast cells were visualized in tissue sections stained with GMS but, at first, the fungal species could not be determined. The multi-budding yeast elements consistent with *Paracoccidioides* were only identified by means of a more thorough examination of serial slides.

Table. Summary of clinical and laboratory findings of 12 cases of Paracoccidioidomycosis due to small forms of *Paracoccidioides*

Case No[Ref]	Sex, Age	Clinical findings	X-ray film	Associated conditions	Histopathologic findings	Mycology diagnosis	ID	Treatment	Outcome
1	F, 64	Dyspnea, asthenia, adynamia, thoracic pain	Bilateral fibronodular infiltrates	Menopause, smoker	Mycotic granulomas	Lung biopsy: GMS + Culture +	+	Ketoconazole*	Recovery
2	F, 57	Anorexia, cough, dyspnea, wheezing	Bilateral fibronodular infiltrates	Menopause (with hormone therapy), smoker	Non-necrotizing granulomatous interstitial pneumonia	Lung biopsy: GMS +	+	Itraconazole	Recovery
3	M, 33	Dyspnea, cough, asthenia, headache, thoracic pain	Bilateral fibronodular infiltrates	Smoker	Granuloma, necrosis and neutrophilic exudate	Lung biopsy: GMS + Sputum +	+	Itraconazole	Recovery
4	M, 68	Asymptomatic	Solitary nodule	Smoker, diabetes, SAH	Granulomas with dystrophic calcification and central necrosis	Lung biopsy: GMS +	-	Surgery	Recovery
5	M, 54	Dysphagia, sore throat, weight loss, cough, asthenia, oropharyngeal ulcerated lesion	Bilateral fibronodular infiltrates and cavitated lesion (7.0 cm)	Smoker, alcoholism, SAH, COPD, tuberculosis	Ulcerative granuloma in squamous mucosa	Oropharynx biopsy: GMS +	-	Itraconazole	Recovery
6	M, 45	Cough, dysphonia, anorexia, asthenia, fever, night sweats, dyspnea	Bilateral fibronodular infiltrates	Smoker	Squamous epithelial hyperplasia and mixed inflammatory infiltrate with giant cells	Larynx biopsy: GMS + Sputum +	+	Sulfadiazine Ketoconazole*	Reactivation of disease 15 years later Recovery

7[20]	M, 59	Cough, anorexya, dyspnea, weight loss	Atelectasis of the lung by bronchial obstruction	Smoker, lung carcinoma	Granulomas with necrosis and one area of calcification	Lung biopsy: H&E +	ND	Pneumonectomy	Recovery
8[12]	M, 53	Progressive dyspnea, asthenia, weight loss, productive cough	Bilateral fibronodular infiltrates	SAH, smoker	Confluent epithelioid granulomas	Lung biopsy: GMS + Sputum -	+	Sulfadiazine	Recovery
9[21]	M, 48	Cough, fever	Consolidation, calcified nodule	Hodgkin's disease, smoker	NA	Lung biopsy: GMS +	ND	Sulfadiazine	Recovery
10[22]	M, 59	Dysphonia	Many calcified nodules scattered in both lungs	Lung carcinoma, smoker	Calcified nodule	Larynx biopsy: GMS + Lung biopsy: GMS +	+	Pneumonectomy Sulfadiazine	Recovery
11[23]	M, 63	Productive cough	Multiple nodules, some with cavities	Smoker, COPD	Necrotizing granulomas	Sputum + Lung biopsy: GMS + Sputum -	ND	NA	Recovery
12[18]	M, 64	Asymptomatic	Solitary, ovoid nodule (1,5 cm)	Smoker	Necrotizing granulomas	Lung biopsy: GMS +	ND	Surgery	Recovery

M indicates male; F, female; SAH: Systemic arterial hypertension; COPD: chronic obstructive pulmonary disease; ID: immunodifusion; GMS: Grocott-Gomori's methenamine silver stain; ND: not done; H&E: hematoxylin and eosin; NA: not available; Ref: references.
* Ketoconazole is no longer used to treat paracoccidioidomycosis. Currently, itraconazole is considered the standard treatment for mild and moderate clinical forms of PCM.

Six patients showed positive reactions to a *Paraccoccidioides* antibody in the immunodifusion test. Two cases were negative. Among the five patients that had sputum examinations, three cases were positive. A fungal culture performed with one patient's biopsy sample (Case 1) yielded a positive result.

In chest X-rays, six patients presented bilateral fibro-nodular infiltrates, and one also had a cavity (Case 5). Two patients had solitary nodules (Cases 4 and 12); two had multiple nodules, calcified (Case 10) or with cavities (Case 11); one patient had consolidation and a calcified nodule (Case 9); and one patient had atelectasis with bronchial obstruction by a neoplasm (Case 7).

For the treatment of PCM, eight patients received medication (ketoconazole, n=2; sulfadiazine, n=4; itraconazole, n=3) and four underwent surgery.

REPRESENTATIVE CASE

A 64-year-old woman was referred and admitted to the Santa Casa Hospital with a chronic cough. She had been a smoker for more than 30 years (two packs of cigarettes daily). Physical examination on admission revealed that her blood pressure was 140/80 mmHg, pulse rate was 96 beats/min., respiratory rate was 16 breaths/min., and temperature 37.7°C. The chest roentgenogram revealed bilateral infiltrates mainly towards the central and upper fields of the lungs and emphysema at the bases (Figure 1). An open pulmonary biopsy was performed. Microscopic examinations of lung tissue revealed a granulomatous inflammation (H&E) and several small yeast forms (Figure 2A). The tissue sections and serum were subsequently sent to the Center for Disease Control and Prevention (CDC) in Atlanta, GA. In the serological tests for antibodies both immune-diffusion and complement fixation were positive, one line of precipitation and titers of 1:32, respectively. A fungal culture of lung tissue was performed and *Paracoccidioides* species was isolated (Figure 2B). Finally, for confirmation, an inoculation of the patient's lung tissue culture in a guinea pig revealed the characteristic forms of *Paracoccidioides* species (Figure 3). The patient was treated with ketoconazole at a daily oral dose of 100 mg, which produced remission of symptoms in one month. Following an uneventful course in the hospital, the patient was discharged, and therapy continued for one year.

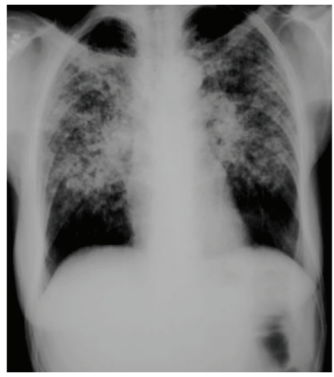

Figure 1. Chest X-ray showing bilateral infiltrates mainly towards the central and upper fields.

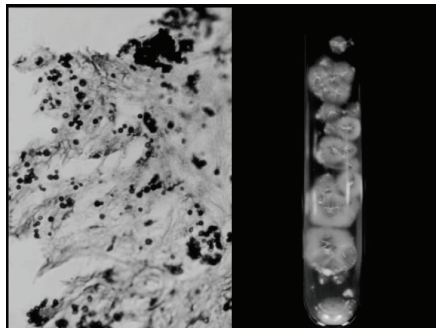

Figure 2. A. Methenamine silver-stained tissue section of lung revealing small forms of *Paracoccidioides* (GMS, 400x). B. Sabouraud dextrose agar with mycelial colonies of *Paracoccidioides* from lung biopsy.

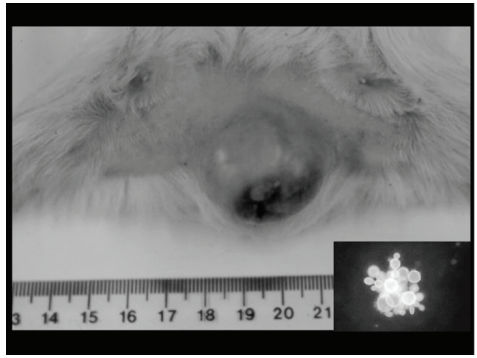

Figure 3. Tissue specimen of a guinea pig's testicle. In the inset, the characteristic multiple budding forms of *Paracoccidioides* (Calcofluor white stain 400x).

DISCUSSION

The tissue reaction to *Paracoccidioides* is similar to those of other systemic mycoses, that is, granulomatous or mixed granulomatous and suppurative infiltrates (El-Zammar & Katzenstein, 2007; Queiroz-Telles & Escussiato, 2011). At times, pathologists may observe fungi occurring in very different circumstances, such as fibrous, cretified and even calcified encapsulated necrotic nodules (Angulo-Ortega, 1972).

In infected tissues, the size, number and form of the organisms vary considerably according to the organ involved, the duration of infection and host conditions (Camargo & Franco, 2000). Small forms of *Paracoccidioides*, as observed in this report, can appear in older patients and in women.

The reasons for the occurrence of small forms of *Paracoccidioides* are still unknown, but they may be related to a host immune response. If the immune response is effective, with granuloma formation, it prevents the proliferation of the fungus (Camargo & Franco, 2000), which may be the reason why only a few multi-budding forms were seen in this series of cases. In women, estrogens inhibit mycelium-to-yeast transformations of *Paracoccidioides*, which provides an explanation for the resistance of females to the disease (Restrepo et al, 1984; Santos et al, 2004). Such inhibition could effectively reduce the propagation of the initially inhaled inoculum, improving the host's ability to prevent the infection's progression, or a delay in *Paracoccidioides* transformation might allow females to generally develop an immune response (Restrepo et al, 1976).

Paracoccidioides infections may remain dormant for very long periods, after which they can be reactivated. Quiescent, residual or latent foci remain in the lungs after involution of the primary infection, as calcifications, encapsulated necrotic nodules or fibrous nodules. In these circumstances, *Paracoccidioides* appears only very sporadically in its characteristic multiple budding forms. Instead it is seen most often in its small form, either without any buds or with just a single one (Almeida & Lacaz, 1940; Angulo & Pollack, 1971; Angulo, 1975; Figueiredo, 1954; Gonçalves et al, 1988; Londero & Chandler, 1997; Melo & Londero, 1983; Restrepo et al, 1976).

The identification of fungi in tissue sections is an important component of the laboratory diagnosis of mycoses, because they are usually large and morphologically distinct. After the routine H&E stained tissue sections, however, special staining may be required to identify some fungal species.

Small forms of *Paracoccidioides*, seen infrequently, may be confused with *H. capsulatum* (Angulo & Pollack, 1971; Queiroz-Telles & Escussiato, 2011), especially when they are intracellular. In this case, a GMS stain must be made; if it is *Paracoccidioides*, the yeast elements are less uniform in size than in *H. capsulatum* and, on examination of many sections in series, a few large elements, with or without buds, may be detected. This difficulty is present

more frequently in old, encapsulated, or necrotic foci. In these latter cases, the distribution of the fungi is helpful for orientation. In cases of PCM the fungi are located in the periphery of the lesion as well as in its center and, principally, in the limit between the capsule and the necrotic area. In histoplasmosis the fungal distribution is more central than peripheral (Angulo & Pollack, 1971; El-Zammar & Katzenstein, 2007; Queiroz-Telles & Escussiato, 2011).

The medium-sized forms of *Paracoccidioides* may be confused with *Cryptococcus* sp. in encapsulated necrotic lesions. Here distinguishing the species is easier: using Mayer's Mucicarmin or a Fontana-Masson stain, the mucinous capsule of the fungus or the melanine in the wall of the fungus are made visible (Gazzoni et al, 2009; Londero & Chandler, 1997).

Moreover, other methods can and should be used to complement the diagnosis of PCM, such as immunodifusion and, particularly, fungal culture. To ensure performing fungal culture, clinicians and surgeons should be aware of the differential diagnosis and be trained to avoid placing all samples in formaldehyde.

Molecular diagnosis relying on polymerase-chain reaction (PCR) and nucleic-acid hybridization, although still at early stages of application to routine diagnosis of *Paracoccidioides*, has triggered the development of techniques for its improved specific detection, thus contributing to epidemiological studies as well.

With prompt diagnosis the prognosis of PCM is usually good, with recovery in most cases.

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