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LETTER TO EDITOR

Paecilomyces formosus Infection in an Adult Patient with Undiagnosed Chronic Granulomatous Disease

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To the Editor,

Chronic granulomatous disease (CGD), an inherited disorder of granulocyte function caused by a failure of intracellular superoxide production, normally presents as severe recurrent bacterial and fungal infections in the first years of life [1, 2]. These recurrent infections occur at epithelial surfaces in direct contact with the environment such as the skin and the mucosal surfaces of the lungs and gut. The majority of affected individuals are diagnosed before the age of 2 years, although patients may remain undiagnosed until adulthood despite the early onset of the symptoms. Lymphadenitis is the most common presenting feature, followed by skin abscesses, pneumonia, and hepatomegaly [3].

The pattern of pulmonary manifestations occurring during adulthood in CGD patients is not well established. Improvements in life expectancy enable most patients to reach adulthood and CGD in adults may be more common than previously assumed [3]. Clinical observations in CGD patients suggest a higher susceptibility to autoimmune diseases, in particular lupus, idiopathic thrombocytopenic purpura (ITP), and rheumatoid arthritis [4]. These recurrent infections occur mostly at epithelial surfaces in direct contact with the environment such as the skin and the mucosal surfaces of the lungs and gut, but can also affect deep tissues as the liver or bones [4].

Infections typically involve the lung (pneumonia), lymph nodes (lymphadenitis), liver (abscess), bone (osteomyelitis), and skin (abscesses or cellulitis) while granulomas typically involve the genitourinary system (bladder) and gastrointestinal tract (often the pylorus initially, and later the esophagus, jejunum, ileum, cecum, rectum, and perirectal area) [5]. The current mortality rate is 2–5% per year [6]. In the absence of prophylaxis with antibiotics and/or interferon gamma, CGD

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patients have severe catalase-positive bacterial and fungal infections about once a year [7, 8]. Hematopoietic stem cell transplantation (HSCT) is now considered as the gold standard for treatment of CGD [8–10].

CGD infections are often caused by a characteristic group of pathogens, including *Staphylococcus aureus*, *Serratia marcescens*, *Burkholdheria cepacia* complex, *Nocardia* spp., and *Aspergillus* spp. [7, 8, 11]. The nitroblue tetrazolium (NBT) test, a simple test for CGD diagnosis, was previously useful for carrier detection [10] but has largely been superseded by the dihydrorhodamine-1,2,3 (DHR-123) assay as a rapid and sensitive assay for CGD diagnosis [12–14].

An 18-year-old female was referred to the Masih Daneshvari Hospital, a center for pulmonary diseases (Tehran, Iran) complaining of cough, dyspnea, and fever for 5 weeks prior to admission. Her medical history revealed the presence of thrombocytopenia at 9 years of age, which was treated as ITP for 4 years with irregular courses of intravenous immunoglobulin (IVIG) and prednisolone which resulted in complete remission. On admission, her height and weight were below the third percentile for her age. We were unable to determine whether this was due to a complication of CGD, the long-term use of glucocorticosteroids in the treatment of her CGD, or the presence of an unknown genetic condition related to being born to a consanguineous family or a combination of all three factors. A complete physical examination revealed bilateral fine crackles. Chest X-ray showed bilateral diffuse pulmonary infiltrations, and a high-resolution CT scan of the lungs was performed revealing mild bronchiectasis and a reticulonodular pattern (Fig. 1a, b). Blood evaluation revealed the following parameters: the WBC count was 28,670 cells/μl with 84% neutrophils, 6% lymphocytes, 5% monocytes, 3% B cells, and 2% eosinophils. The hemoglobin level was12.7 g/dl; platelet count was 417,000/μl; the erythrocyte sedimentation rate was 70 mm/h; and the blood urea nitrogen (BUN) and creatinine levels were within the normal range. Viral PCR testing of sputum for influenza and respiratory syncytial virus was negative. Lactate dehydrogenase and beta-D glucan were elevated at 845 units/l (normal 135–376 U/l) and 424 pg/ml (normal <60 pg/ml), respectively. This suggested the diagnosis of the presence of an infectious agent most likely mycosis. The search for auto-antibodies such as antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibody (ANCA), cytoplasmic anti-neutrophil cytoplasmic antibodies (c-ANCA), rheumatoid factor, anti-double stranded DNA, and anti-Scl-70 were all negative.

Sputum smear and culture tests for tuberculosis were negative. Tests for hepatitis B surface antigen (HBsAg), antibodies to HBs, and antibodies to HIV and to hepatitis C virus were also negative. IgG, IgM, IgE, IgA, total complement hemolytic activity, and C3 and C4 levels were all within normal limits.

The results of neutrophil chemotaxis testing and flow cytometric analysis of PBMCs including CD3 (total) T cells, CD4 (helper) T cells, CD8 (cytotoxic) T cells, CD19 (B cells) cells, CD56 (natural killer cells, and adhesion molecules (CD18, CD11a, CD11b, and CD11c) on lymphocytes, neutrophils, and monocytes were also normal.

At this time bronchoscopy, bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBLB) were performed. Analysis of the BAL for bacteria, viral, and fungal agents was negative using bacterial gram staining, PCR and fungigram staining, and KOH and culture of BAL samples, respectively. Grocott's methenamine silver staining (GMS) for fungi was not performed. Serum and BAL galactomannan (GAM) tests were also negative. Pathologic examination of the lung tissue with hematoxylin and eosin staining determined a granulomatous inflammation (Fig. 2). Unfortunately, tissue samples were not assessed for bacterial culture. NBT and DHR 123 tests were performed and results were consistent with CGD (Fig. 3). The patient underwent appropriate antibiotic therapy consisting of vancomycin, meropenem, co-trimoxazole, and voriconazole.

Ten days after treatment, the patient deteriorated with a high fever (39.5 °C) and hypoxemia (blood O_2 saturation 75%). A second bronchoscopy and TBLB procedure were performed and the culture of lung tissue for fungal agents was performed. The TBLB specimen was negative for fungal staining; however, culture of lung tissue yielded >200 colonies of a mold with yellowish-gray colonies with a black

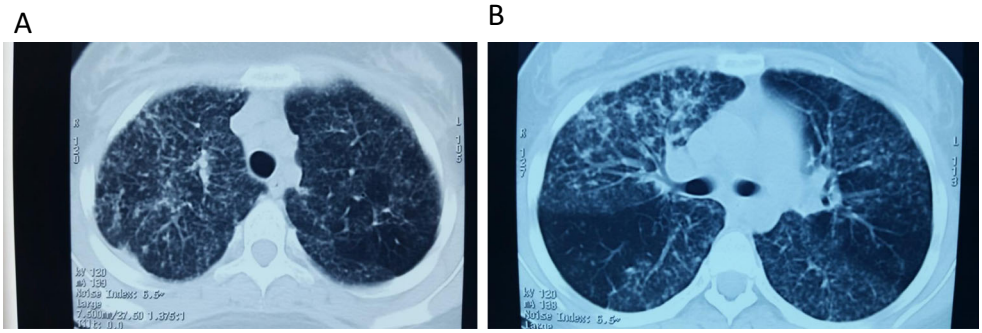

Fig. 1 HRCT of the lungs: coarse interstitial reticulation associated with fibrotic areas, fine nodules, and scattered lobular consolidations (**a**, **b**)

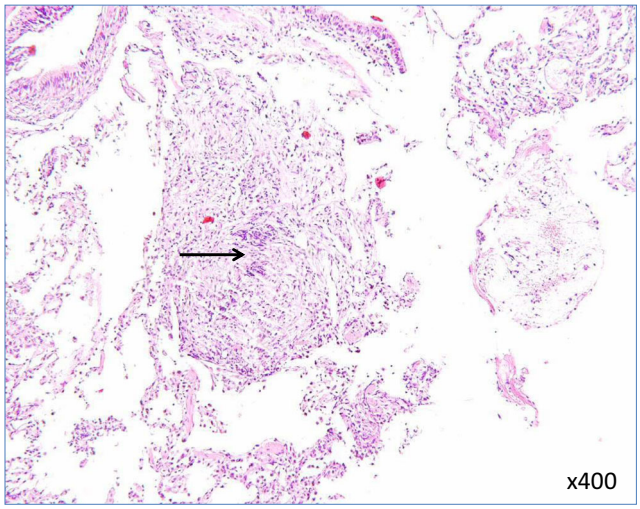

Fig. 2 Lungs biopsy of hematoxylin and eosin (H&E). Lung sections were stained by H&E and show granulomatous inflammation (*arrowed*)

reverse after growth on blood agar (BA) and sabouraud dextrose agar (SDA) plates (Fig. 4a).

Microscopic analysis revealed branching solitary phialide with ellipsoidal conidia with long chain arrangement and many chlamydospores which mostly resemble *Botryotrichum* species but during subcultures on potato dextrose agar (PDA) and SDA, phialides typical of *Paecilomyces* species appeared (Fig. 4b). In addition, many yellowish-brown chlamydospore were present with smooth walls. Repeated transfer on PDA and on water agar did not detect any ascospores. This organism was sensitive to amphotericin B (minimum inhibitory concentration, MIC = ~0.5), caspofangin (MIC = ~1), and voriconazole (MIC = 0.125) in a fungigram assay; however, no PCR confirmation of the fungal species was performed. Fungal DNA was extracted using cetyltrimethylammonium bromide (CTAB) buffer [15], and amplification and sequencing of the rDNA internal transcribed space region (ITS)1 and ITS4 and of the 5.8 rRNA regions of the nuclear ribosomal RNA gene cluster was performed [16].

The sequences were aligned with those in the EMBL GenBank database using Fasta 2 and found to be 99% identical to that of *Paecilomyces formosus* (EMBL GenBank fungal library accession no. KC157764) and *Byssochlamys spectabilis*. There was 100% homology of the 5.8S rDNA ITS domain sequences with *Paecilomyces formosus* CBS121584. Finally, identification of the fungi as *Paecilomyces formosus* was confirmed by acid production after 5 days under the colony on creatine agar [17]. As a result of this analysis, her treatment was changed to liposomal amphotericin B and caspofungin. After 6 weeks of treatment, the patient made a full recovery from an infectious standpoint and was sent home on prophylactic doses of co-trimoxazole and voriconazole.

Discussion

CGD is an inherited disease in which the oxidative burst in granulocytes is suppressed. This defect leads to recurrent

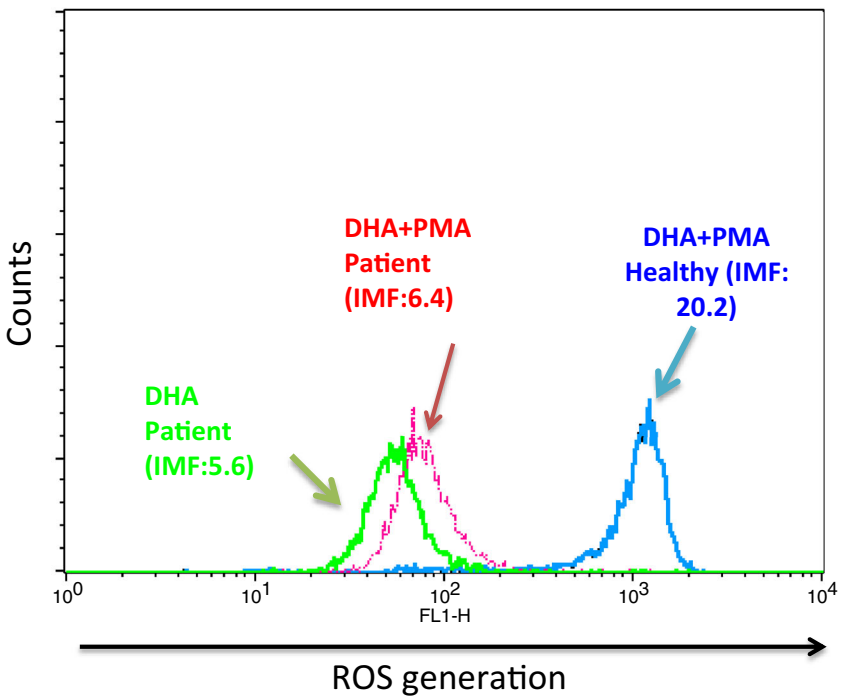

Fig. 3 Flow cytometry of DHR123 reaction. Quantification of the DHR123 reaction. Patient and healthy control cells were incubated with DHR123 (375-ng/ml final concentration), with or without PMA (final concentration = 100 μg/ml) stimulation, and ROS generation was assayed by FACS analysis. The mean fluorescent intensity (MFI) of the following groups are indicated in the figure: *green lines* represent DHR123-labeled cells from unstimulated healthy controls and patient cells, *blue line* represents PMA-stimulated DHR-labeled healthy control cells, and *red line* represents DHR123-labeled and PMA-stimulated patient cells

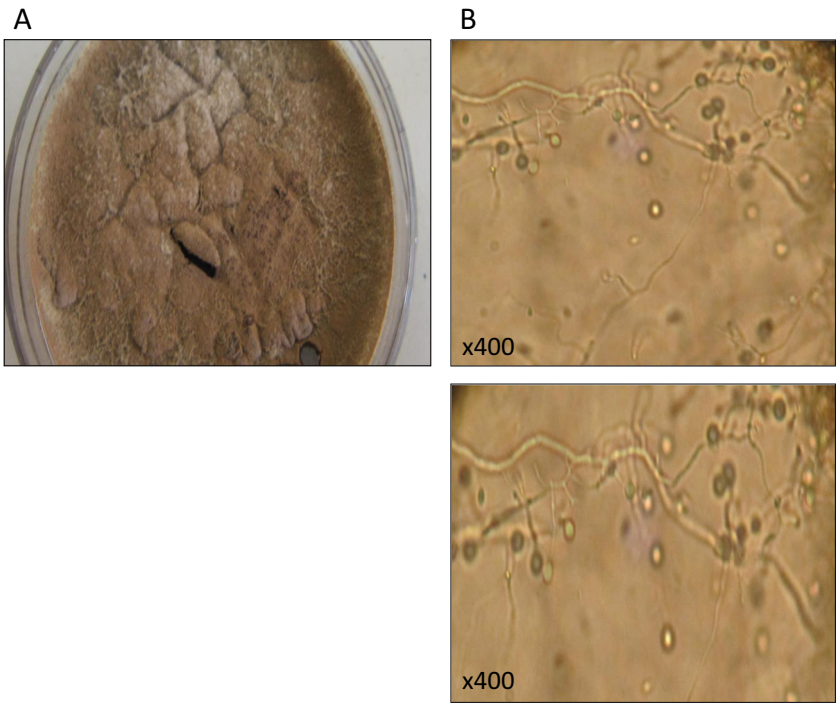

Fig. 4 Culture of lung specimens obtained by bronchoscopy. **a** It shows fungal growth after sterile culture. **b** The characteristics of this fungal growth were specific for Mycelium of *Paecilomyces formosus*

bacterial and fungal infections, particularly with pathogens that are catalase-positive such as aspergillus species, *Staphylococcus aureus* and *serratia* species, *Nocardia* species, and *Burkholderia cepacia* [6–8]. CGD is a primary immunodeficiency disorder, and most cases are diagnosed at childhood [14]. The X-linked forms are more common, but autosomal recessive forms (AR-CGD) have also been described [6]. Most patients with CGD receive this diagnosis during the first years of life. Some older patients with CGD have also been described. In such patients, mild phenotypes are considered to be the reason for the unusually late manifestations since AR-CGD has a milder phenotype than X-linked CGD [18]. Interestingly, the autosomal recessive form of CGD is the most common form within Iranian families (87.1%) [19]. In contrast, the most common inherited pattern in the USA [6] and Europe [20, 21] is XL-CGD with incidences >57%. The diverse inheritance pattern observed in Iran provides novel information about CGD [22–24]. This discrepancy may be partly accounted for by the high rate of consanguinity in Iranian families [25]. Interestingly, the current CGD patient is the fourth child of consanguineous parents (cousins). Four members of the family were evaluated for CGD by NBT assays and only one brother had thrombocytopenia with abnormal NBT suggestive of CGD.

To our knowledge, this is the first report of *Paecilomyces formosus* infection in the context of CGD. In this regards, it previously indicated that *Paecilomyces variotii* is a commonly occurring species in air and food but is also associated with many types of human infections and is among the emerging causative agents of opportunistic mycoses in immunocompromised patients. Typically, *Paecilomyces* spp. rarely cause infections in humans [26–28], and if these fungi are detected in blood, urine, or cerebrospinal fluid cultures, they are considered as contaminants [26]. Whereas *Paecilomyces lilacinus* and *Paecilomyces variotii* may cause opportunistic infections and able to affect the eyes and skin in immunodeficient patients [27, 29]. There are some previous reports of *Paecilomyces* spp. infections in adult patients with immunodeficiency or post-traumatic disorders but there are few reports of such infections in pediatric patients [26, 27, 30, 31].

Chronic *Paecilomyces fusarium* infection in an adult patient with undiagnosed chronic granulomatous disease has been previously reported by Mansouri and colleagues in an adult CGD patient [32]. Thus, despite its current relative rarity in older patients, the diagnosis, management, knowledge, and outcome of CGD are rapidly changing. Greater understanding of the biology of CGD and its treatment in older patients requires concomitant advances in medicine, basic science, and genetics.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict interest.

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