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Enhancement in serum (1–3)-β-D-glucan level by cutaneous alternariosis: A case report and literature review

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ABSTRACT

Contamination with the fungus *Alternaria* spp. is often considered to have originated from laboratory sources, which occasionally causes infection in immunocompromised patients, termed as phaeohyphomycosis. Here, we have reported a case of cutaneous alternariosis caused by *Alternaria alternata*. This diagnosis was based on microscopic examination and mycological culturing of patient's vesicular lesions, with the use of 5 molecular markers (namely, ITS, ATPase, Actin, rpb2, and tef1) for strain identification. We noted that *Alternaria* infection caused an increase in the serum level of (1–3)-β-D-glucan (BG) in the patients. To the best of our knowledge, no such finding has been reported in previously in the literature.

1. Introduction

More than 280 subspecies of *Alternaria* species have been identified, most of whom do not cause human infection; however, *Alternaria* infection can be fatal in immunosuppressed people [1]. The most commonly reported *Alternaria* spp. in connection with human infection are *A. alternata*, *A. tennuissima*, and *A. chartarum* [2–4]. The existing literatures mainly focuses on the clinical manifestations and treatment of *Alternaria* infection, with a few involving auxiliary examinations such as the (1 → 3)-β-D-glucan antigen (BG) assay. In the present report, we noted that the BG assay results were significantly abnormal for *A. alternata* infection, which may be a new auxiliary diagnostic basis for *A. alternata* infection.

2. Materials & methods

2.1. Mycological culturing

The patient's vesicle fluid was extracted with a syringe, the blister liquid was centrifuged at 3000 rpm for 10 min, and the sediment was inoculated on Sabouraud's agar medium (SDA) and incubated at 28 °C, daily observation.

2.2. BG assay

BG assay was performed using a kit (Dynamiker Biotechnology [Tianjin] Co., Ltd.) based on spectrophotometry for the quantitative detection of BG in the human serum. The patient's blood sample was collected and centrifuged at 3000 rpm for 10 min, the serum obtained was then tested immediately (if the test cannot be performed immediately, glucan-free EP tubes can be used to store 1-mL of the serum samples at 2–8 °C before assay, but the storage should not be extended beyond 3 days). The assay was performed in strict accordance with the manufacturer's instruction. The value of <70 pg/mL indicated a negative result and ≥95 pg/mL indicated a positive result. A value of ≤70 pg/mL and <95 pg/mL indicated an inconclusive result, with a suspicion of invasive fungal infection requiring repeat sampling and assay.

2.3. Isolate identification

Five phylogenetic markers were used for strain identification, namely, ITS, ATPase, Actin, rpb2 and tef1. The primer design and amplification conditions of these 6 markers were referred from the relevant existing literature [5,6]. DNA extraction, primer synthesis, and amplification product sequencing were completed by the BGI Tech Solutions (Beijing Liuhe) Co., Limited. The sequencing outcomes of ITS was deposited in the NCBI's GenBank nucleotide database, with the GenBank accession numbers of MW284844. The results from this 5

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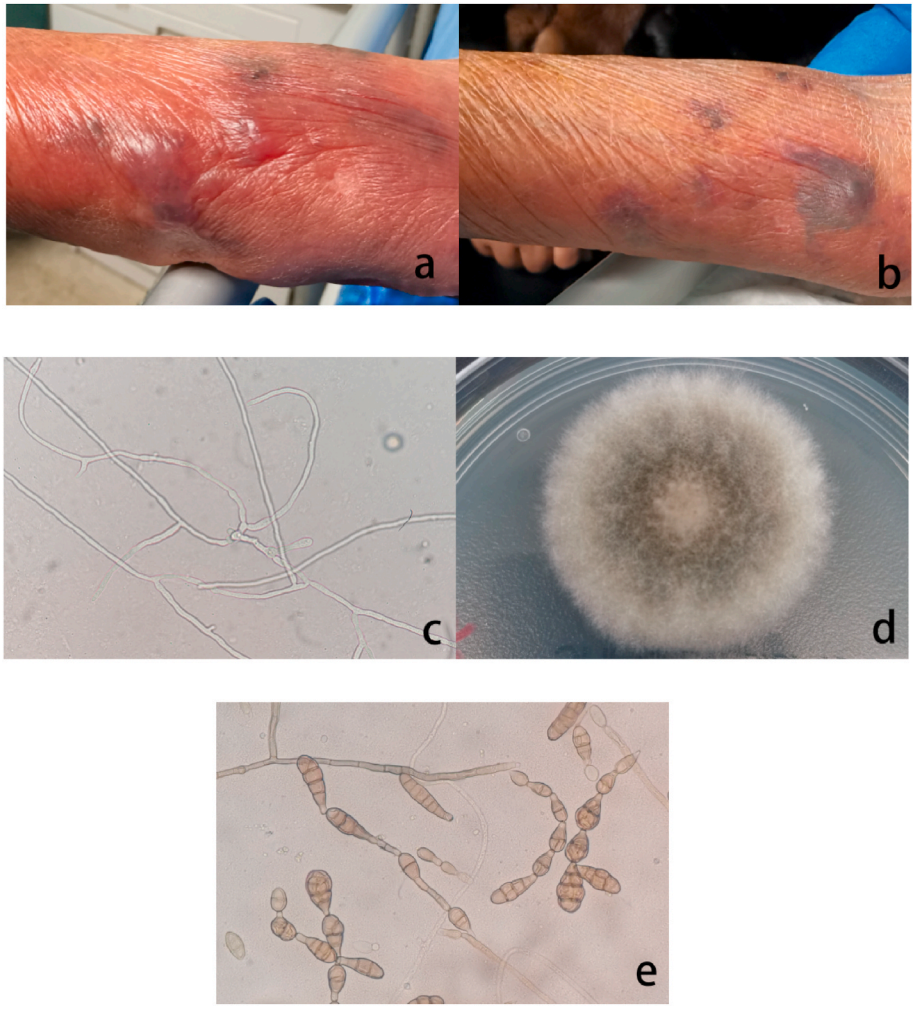

Fig 1. a: The blisters on patient's forearm before treatment with antibiotics existed in single or multiple forms and were easily fused together; the contents were colorless and slightly turbid liquids. **b**: After oral voriconazole therapy, the blisters disappeared and were replaced with brown scar tissues. **c**: The blister fluid was extracted for microscopic examination. The hyphae were light green, obtusely round at the top and slender at the end. 10% KOH, ×400. **d**: After culturing for 3 days, the colony appeared round, grayish-green, margin regular, and fluffy on SDA at 28°C. **e**: The conidia are light brown, inverted clavate, with a brick lattice separated longitudinally and horizontally, with beak-like protuberances at the top and arranged in long chains or branches, as observed after 3 days of culturing on SDA under ×400 magnification.

phylogenetic markers blast sequence alignment revealed that our strain highly matched *A. alternata*.

2.4. Case report

The case patient was an 83-year-old man admitted for cough and sputum on admission. He also presented with localized skin edema and blister formation in both the upper extremities with moderate edema in both the lower limbs. The patient was diagnosed with coronary heart disease and osteoporosis 10 years ago. His laboratory findings revealed a white blood cell count of 8.43×10^9/L, a high-sensitivity C-reactive protein level of 73.06 mg/L, a b-type natriuretic peptide level of 59.2 pg/mL, a total protein content of 50.1 g/L, a pre-albumin level of 30.7 g/L, a urea concentration of 13.35 mmol/L, a creatinine level of 152.4 μmol/L, and a creatinine clearance value of 31.3 mL/min. The patient's blood culturing, tuberculin skin test, and interferon gamma release assays were all negative. His chest computed tomography however revealed interstitial changes in both the lungs.

The patient was accordingly administered with cefotaxime, methylprednisolone, albumin, and other symptomatic treatments. After 10 days, his symptoms of lung infection improved, but the blisters in the right upper limb increased significantly. The medication was changed from antibacterial drug to cefoperazone/sulbactam, but the blisters continued to increase (Fig 1a). Next, the blister fluid was extracted for microbial culture; the smear of the blistering fluid drawn showed a large number of fungal hyphae (Fig 1c), and filamentous fungal growth was recorded on culturing on SDA (Figs 1d, 1e). To determine the presence of fungal infections, we conducted repeated mycological culturing and BG assay 2 days later and obtained the same results. After pathogen identification, methylprednisolone therapy was discontinued and voriconazole therapy was initiated. After 5 days, the blisters subsided (Fig 1b), and the patient was discharged with improved symptoms; but he was continued on the oral voriconazole therapy. After 2 weeks, no recurrence of the skin infection of the limbs was noted.

3. Discussion

Phaeohyphomycosis is defined as a chronic infectious condition caused by a heterogeneous group of fungal pathogens, known as dematiaceous fungi, which are characterized by the formation of dark colonies in culture as a result of the production of melanin-like pigment. *Alternaria* is one of the most frequent causative agent of cutaneous phaeohyphomycosis representing an important emerging pathogen in immunocompromised patients [7]. Meanwhile, *Alternaria* is a common saprophytic fungus in the environment that can colonize on the skin and the conjunctiva of normal people and animals [8]. Recent reports have proved that *Alternaria* can not only cause skin infection [9] but also lung infection [10], fungal keratitis [11], and allergic diseases [12], which further increase the susceptibility of influenza viruses [13], thus requiring more attention.

Immunosuppression and damage to the skin barrier are the main pathogenic mechanisms of cutaneous mycosis [14]. Therefore, cutaneous *Alternaria* infection usually occurs in the exposed areas of the body, such as the limbs and faces [15]. Our patient was an old man with

a history of coronary heart disease since the past 10 years with renal insufficiency, hence his immune system may be impaired. Moreover, he received steroid therapy and the infection site was the limbs, which is consistent with the related literature reports. Usually, cutaneous alternariosis is diagnosed by histopathological examination and microbial culturing [3,8,10,16]. In our report, fungal hyphae were detected in the blister fluid collected via aseptic operation, and *A. alternata* was cultivated. Meanwhile, during the treatment of this patient, cefotaxime and cefoperazone/sulbactam were used, but they seemed ineffective, with improvement in the symptoms observed after antifungal treatment, which also proves that *A. alternata* is the causative pathogen.

The clinical manifestations of *Alternaria* dermatitidis infection can be categorized into the epidermic and dermal types. The epidermal type displays scaly infiltrating erythema, while the dermal type displays papules and pustules [2,8,15]. However, our patient demonstrated a blister-like appearance. We thus speculated that this case was of the dermal type, because the patient showed renal insufficiency and retention of water and sodium, which led to the development of subcutaneous edema and mycelium invasion, and fungal infection destroyed the dermis. All these factors resulted in the leakage of tissue fluid into the epidermis and manifested as a vesicular lesion.

Unexpectedly, the value of BG assay in our patient was >600 pg/mL, which has been rarely reported in cases of *Alternaria* infection. The BG assay can be positive in patients with the infection of *Candidiasis*, *Cryptococcosis*, *Aspergillosis*, and *Pneumocystis carinii*, but it is typically negative in patients with *Mucormycosis* or *Fusariosis* [17–21]. However, it is undeniable that there are several false-positive factors in the BG assay, such as intravenous infusion of immunoglobulin, albumin, certain antibiotics, and hemodialysis treatment [22]. These influencing factors should be considered when analyzing their clinical significance. Past studies have shown that the use of albumin is the most important factor leading to false-positive results in BG assay. Its effect on the testing outcomes is statistically significant only when >30 g of albumin was used within 2 days, and it also proved that, even when used within 2 days in case of >30 g of albumin, the diagnostic threshold was set at the level of 203 pg/mL and it showed good diagnostic specificity (84.7%) [23]. Due to severe hypoproteinemia, our patient undertook intravenous albumin therapy before BG assay, but the dosage was 10 g/day, which should have had a minor impact on this assay. The other possible influencing factor involved in this case was the administration of cefotaxime; however, past studies have shown that, at the normal infusion concentrations, cefotaxime could led to the results of BG assay to increase to 153 pg/mL [24], which is far lower than the actual detection value in the present case. Therefore, this case suggests that the value of the BG assay was increased sharply during *A. alternata* infection. It could be potentially used as an auxiliary diagnostic indicator of *Alternaria* infection, although confirming its clinical value warrants further clinical research.

For the selection of *anti-Alternaria* drugs, the literature reports vary considerably. For instance, in 2008, F. J. reported that itraconazole was the most effective antifungal drug, with reports showed an efficiency of even 90% [8]. However, some other reports proved that itraconazole was only approximately 60% effective [25,26]. However, a literature in 2020 reported that the use of itraconazole alone showed an efficiency of 41.94% [27]. Although this case can be counted in the literature to differ between individuals and infected sites, the resistance of *Alternaria* to itraconazole should also attract clinical attention. In addition, other antifungal drugs such as voriconazole [11], amphotericin B [16,28], fluconazole [25], isaconazole [29], and posaconazole [10] have been reported to be successful in treating fungal infections. Our report thus also proves that voriconazole is an effective regimen.

In conclusion, *Alternaria* often causes infection in immunocompromised people. In addition to histopathological examination and microbial culturing, the serum BG level detection can be used as a potential auxiliary diagnostic, albeit further research is required to validate and confirm its significance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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