

Case report

Hypersensitivity pneumonitis caused by *Fusarium napiforme* in a home environment

Background: We report a case of hypersensitivity pneumonitis (HP) in a 17-year-old male student caused by *Fusarium napiforme* found in his home environment.

Methods: The patient was diagnosed according to history, chest radiograph, spirometry, high-resolution chest CT, and transbronchial lung biopsy. To identify the causative agent, cultured aeromolds were collected by the open-plate method. From the main fungi cultured, fungal antigens were prepared, and immunoblot analysis with the patient's serum and each fungal antigen was performed.

Results: Five fungal species were isolated from the patient's home. Immunoblotting analysis with the patient's serum demonstrated more than 10 IgG-binding fractions to *F. napiforme* extract only, while little binding was noted with the other fungal antigens.

Conclusions: We should be aware that HP may be caused by *F. napiforme* in the home environment.

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Hypersensitivity pneumonitis (HP) is an immunologically mediated lung disease caused by repeated inhalations of organic antigens. HP is most often due to an occupational exposure (farmer's lung [1], sawmill worker's disease [2], maple bark stripper's disease [3]). However, HP can be induced by exposure in a home environment to an avian antigen as in bird fancier's disease (4) and in patients using home ultrasonic humidifiers (5, 6). Contamination of homes by *Trichosporon cutaneum* has been described with Japanese summer-type HP (7). Heated swimming pools and composting waste at home have also been associated with HP (8, 9). In Korea, there have been reports of HP caused by household exposure to *Trichosporum cutaneum* (10) and *Penicillium expansum* (11).

In this paper, we report a case of HP caused by mold exposure (*F. napiforme*) in a home environment, which was confirmed by immunoblot analysis based upon *F. napiforme* isolated from the patient's home. To the best of our knowledge, this is the first report of HP caused by *F. napiforme* in a home environment.

Case report

A 17-year-old, nonsmoking male student was referred with a 2-week history of respiratory symptoms. He experienced dyspnea, coughing, and febrile sensation 2 days after the repair of the basement in his house. His symptoms were worse when he was at home than at school. Initial physical examination showed inspiratory crackles in both basal lung fields. His leukocyte count was 10 900/mm³ (neutrophil: 84.5%, lymphocyte: 10.4%, monocyte: 4.4%, eosinophil: 0.3%). On the chest radiograph, there were diffuse tiny nodular densities and increased interstitial markings. High-resolution chest computed tomography (CT) revealed multiple tiny nodules and ground-glass opacities on both lung fields. Spirometry showed FVC of 1890 ml (41% predicted), FEV₁ of 680 ml (43% predicted), FEV₁/FVC of 89%, MEFR₂₅₋₇₅ of 3.17 l/s (74% predicted), and DLCO of 11.61 ml per min/mmHg (46% predicted). Rheumatoid factor (RF) was 487.0 U/ml, and complement C3 and C4 levels were normal. Serum IgG (1590 mg/dl), IgA (330 mg/dl), and IgM (466 mg/dl) were increased. Skin prick tests with 80 common inhalant and food allergens

showed positive responses to *Trichophyton* spp. (4+). Total IgE by AlaStat (DPC, USA) was 151 IU/ml. Sputum stainings for *Mycobacterium tuberculosis* were negative. The pathologic findings of specimens obtained by transbronchial lung biopsy demonstrated lymphocytic infiltration within the alveolar walls without evidence of epithelioid granuloma, or bacterial or acid-fast bacilli infection. By 2 days after admission, his symptoms were improved dramatically, and 6 days later, he was discharged with antituberculosis medication and an oral corticosteroid. After several hours at home, his respiratory symptoms recurred, worsened progressively day by day, and became severer than at the first admission. He then visited our emergency room again and was readmitted. By 24 h after admission, his symptoms were improved with avoidance of exposure and corticosteroid therapy. Under the diagnosis of HP, he was advised to move to his uncle's house and take corticosteroid therapy for 2 weeks. Clinical symptoms, abnormal findings on chest radiography, and spirometric abnormalities disappeared after 1 month's avoidance of exposure.

Material and methods

Isolation and identification of fungi

Saboraud glucose agar (Difco, Detroit, MI, USA) plates containing 0.06 gm/l of chloramphenicol were left open for 2 h on the floor in the patient's bedroom, living room, and toilet, and then incubated at 28°C and 37°C for 10 days. Colonies appearing on the plates were subcultured and identified morphologically.

Crude antigen preparation

Each fungal isolate was cultured in saboraud glucose agar for 3 days and incubated in Czapek-Dox broth media (Sigma, St Louis MO, USA). Incubated broth media were kept at 37°C for 4 days on a gyratory shaker. Fungal mycelia were separated from the broth media by passing through Whatmann filter paper. Mycelial extract was separated in liquid nitrogen containing sea sand. After shaking for 12 h at 4°C in phosphate-buffered saline containing 0.1% Triton X-100, the extract was separated again ultrasonically. The supernatant obtained through centrifugation and dialysis against distilled water was lyophilized.

SDS-PAGE and immunoblotting

SDS-PAGE was performed by the method of Laemmli (12). Mycelial extract antigens were dissolved in a sample buffer (Novex, San Diego, CA, USA) and boiled for 5 min. Standard markers (3–185 kDa) (Novex, San Diego, CA, USA) and antigens were applied to a Novex precast NuBis-Tris gel (4–12%) for the separation of fungal antigens. Electrophoresis was

performed with a Novex X cell II mini-cell for 40 min at 200 constant voltage. The gel was fixed and stained with 0.1% Coomassie brilliant blue. By the method previously described by Tsang et al. (13), electroblotting was carried out for 60 min at 30 V in Tris-glycine transfer buffer with 10% MeOH with a Novex X cell II blot module. After transfer, the nitrocellulose membrane was blocked with Tris-buffered saline (TBS) containing 5% skim milk. The patient and control sera were diluted to 1:150 v/v with 5% skim milk/TBS. The membrane was then incubated with the patient and control sera for 1 h at room temperature. It was then washed with TBS. Peroxidase-conjugated goat anti-human IgG was diluted to 1:1000 with 5% skim milk/TBS and used as a secondary antibody solution. The membrane was then incubated with the secondary antibody for 1 h at room temperature and washed again as above. A substrate solution (0.05% 4-chloronaphthol, 0.01% hydrogen peroxide) was applied to the membrane until the positive bands appeared.

Results

Fungal isolation and culture

F. napiforme was the predominant isolate found in the air of all sites of the patient's home. A few colonies of *Aspergillus niger*, *A. nidulans*, *Trichoderma* spp., and *Curvularia* spp. were also present.

Gel electrophoresis and immunoblot analysis

To determine the protein components of five fungal extracts (*A. niger*, *A. nidulans*, *Trichoderma* spp., *F. napiforme*, and *Curvularia* spp.), the extracts were analyzed by a 4–12% gradient SDS-PAGE. Fig. 1 reveals the resolution of the five fungal species into respective bands. Fig. 2 demonstrates the binding of specific IgG in patient and control sera on the blotted nitrocellulose membrane. More than 10 bands ranging from 26.7 to 185 kDa were bound to *Fusarium* spp. extracts, while no specific bindings were found in other fungal extracts.

Discussion

Species of *Fusarium*, the largest genus of Tuberculariaceae, occur saprophytically or parasitically on many crops, including fruits and vegetables. They have been commonly considered a contaminant, but are known to be a relatively common agent of mycotic eye infections, and are occasionally involved in skin and nail infections and in systemic infections of severely debilitated hosts (14). Melcher et al. (15) reported *F. napiforme* as the cause of disseminated hyalohyphomycosis. There has been no case report of HP due to *F. napiforme* in a home environment.

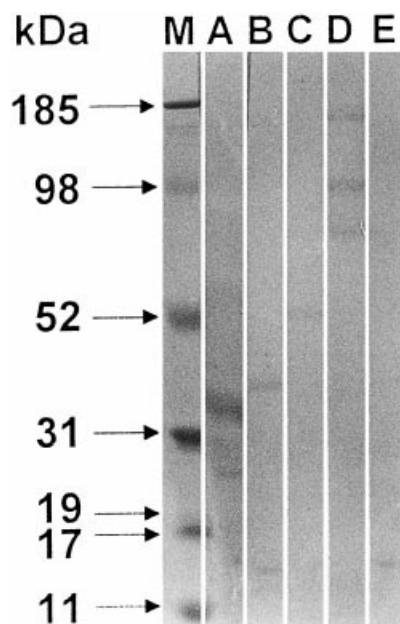


Figure 1. SDS-PAGE of five fungal species. A) *Aspergillus niger*, B) *A. nidulans*, C) *Trichoderma* spp., D) *Fusarium napiforme*, E) *Curvularia* spp.

The diagnosis of HP requires a constellation of clinical, radiographic, physiologic, pathologic, and immunologic criteria, each of which is rarely pathognomic alone. In general, the environmental history often provides a clue as to the potential etiologic agent. Episodes of recurrent fever with breathlessness, cough, and malaise that follow a home environmental exposure are typical of extrinsic allergic alveolitis. The chest roentgenogram showed ground glass-like appearances, CT showed interstitial pneumonia, and spirometry showed restrictive ventilatory impairment with decreased diffusion capacity. Moreover, the pathologic findings of a specimen obtained by transbronchial lung biopsy showed interstitial lymphocytic infiltration. All of these characteristics are consistent with the diagnosis of HP. Inhaled mold spores could provoke production of specific antibodies. The detection of these antibodies plays an important role in the diagnosis of HP. Although Ouchterlony's double immunodiffusion is the classical test to investigate specific antibodies, other studies such as counterimmunoelectrophoresis, radioimmunoassay, ELISA, crossed radioimmuno-

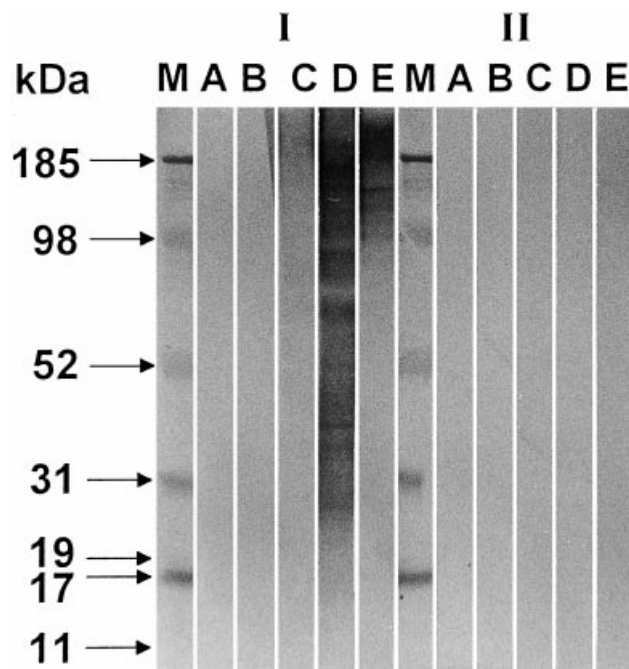


Figure 2. Findings of IgG immunoblotting with sera of patient (I) and healthy control (II). Molecular size marker (M): multicolored standard (Novex, CA, USA); molecular weight: myosin (185 000), phosphorylase B (98 000), glutamic dehydrogenase (52 000), alcohol dehydrogenase (31 000), carbonic anhydrase (19 000), myoglobin (17 000), lysozyme (11 000), aprotinin (6000), and insulin (3000). A) *Aspergillus niger*, B) *A. nidulans*, C) *Trichoderma* spp., D) *Fusarium napiforme*, E) *Curvularia* spp.

phoresis, or immunoblotting can also be used (16). In this study, five fungal species were isolated from the patient's home environment. Among them, IgG immunoblotting demonstrated specific IgG-binding components to only *F. napiforme* extract. The presence of serum specific IgG antibody to the *F. napiforme* antigen isolated from the patient's home provides additional support for the diagnosis of HP and suggests that *F. napiforme* was the causative agent.

In conclusion, this is a case of HP occurring in the home and caused by the fungus, *F. napiforme*. This case report emphasizes the need to be aware of the clinical presentation of HP after mold exposure in a home environment.

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