

[CASE REPORT]

Occupational Hypersensitivity Pneumonitis in a Japanese Citrus Farmer

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Abstract:

Hypersensitivity pneumonitis (HP) sometimes develops in people working in specific environments. We herein report a case of occupation-related HP in a citrus farmer in Japan. A 66-year-old man developed a fever, dyspnea, and general malaise in March after working near a trash dump filled with moldy tangerines. He presented with leukocytosis, bilateral lung opacities on chest radiographs, and intra-alveolar and interstitial lymphocytic inflammation with fibrotic change on a lung biopsy. His symptoms disappeared after admission and recurred on a revisit to the workplace. Fungal culture and a mycobiome analysis using next-generation sequencing suggested an association with exposure to *Penicillium digitatum*.

Key words: fungi, hypersensitivity pneumonitis, next generation sequencing, occupational hypersensitivity pneumonitis, *Penicillium digitatum*

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Introduction

Hypersensitivity pneumonitis (HP) is an allergic, diffuse pneumonia caused by repeated inhalation of antigenic substances (1). Causal antigens of HP can be classified into six categories: i) fungi/molds, ii) bacteria, iii) animal proteins, iv) plant proteins, v) low-molecular-weight chemicals, and vi) metals (1-3). Bird-related hypersensitivity pneumonitis accounts for 66-68% of all HP in Japan and in other countries, followed by farmer's lungs and HP caused by housing-related fungi, such as summer-type hypersensitivity pneumonitis (4-8). Clinical characteristics of HP differ depending on the type of antigen and the magnitude and duration of environmental exposure.

In some cases, HP develops in association with specific occupations, termed occupation-related HP (OHP). Various types of agricultural workers may develop OHP, such as

farmer's lung (9), greenhouse worker's lung (10), and mushroom worker's lung (11). Molds and fungi are the most common cause of OHP: indeed, 98% of newly-developed OHP in Finland were attributed to molds (3).

We herein report a case with OHP in a citrus farmer possibly associated with exposure to *Penicillium digitatum*.

Case Report

A 66-year-old man who was a current smoker of 30 pack-years was admitted to a hospital due to a fever, dyspnea, and general malaise. He had been working in a citrus farm for decades, where he harvested two varieties of tangerines (one at the end of autumn, and one at the end of winter) and dumped damaged fruits before shipping in a storehouse. In March, he felt dyspnea and malaise 10 days before the admission. Three days later, he developed a fever of 38°C.

Chest X-radiography showed consolidation in the bilateral

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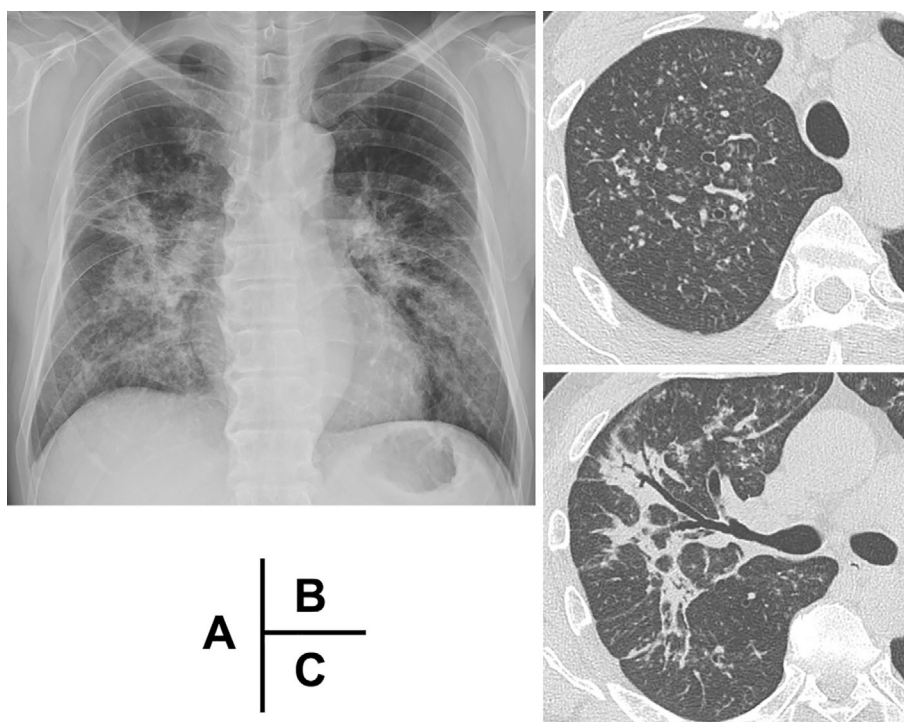


Figure 1. Radiographic findings of the lungs one week prior to the admission. Chest X radiography (A) showed consolidation in the bilateral lungs, and high-resolution computed tomography demonstrated centrilobular nodules (B) and consolidation along with bronchovascular bundles and traction bronchiectasis (C).

lungs, and chest computed tomography demonstrated diffuse, small-nodular shadows, consolidation along with bronchovascular bundles and traction bronchiectasis (Fig. 1). Peripheral blood tests demonstrated leukocytosis ($12,200/\mu\text{L}$) and elevated serum levels of C-reactive protein (CRP, 14.8 mg/dL). His forced vital capacity had decreased from 4.01 L in the previous summer to 2.55 L (74.8% of the predicted value), and the carbon monoxide transfer coefficient had decreased from 101% to 87% of the predicted value. A bronchoscopic examination was performed 5 days after admission, showing increased total cell counts ($4.47 \times 10^5/\text{mL}$) and lymphocytosis (21%) with an increased CD4/CD8 ratio (4.55) in the bronchoalveolar lavage fluid (BALF) from the middle lobe of the right lung. Pathologically, intra-alveolar and interstitial lymphocytic inflammation was observed in the right lung specimen obtained by a transbronchial biopsy, with collagen deposition on Elastica van Gieson staining (Fig. 2). There was no granuloma or aggregation of CD68-positive macrophages.

His symptoms as well as laboratory abnormalities had mostly disappeared spontaneously within 10 days of admission, but radiographic improvement was only partial at this point. When he re-visited the storehouse and his home for 1 hour as a test of environmental exposure, his serum CRP levels gradually increased from 2.0 to 6.0 mg/dL within 5 days. No changes in the symptoms, body temperatures, or radiographic findings were observed. The serum CRP levels decreased and remained negative even after being discharged home while avoiding any visit to the storehouse.

We inspected his house, finding no fungal contamination, duvets, or humidifiers inside the house, but moldy tangerines were found stacked in a trash dump next to the storehouse. Culture of the moldy tangerines demonstrated multiple species of fungi, including *Candida californica*, *Dipodascus geotrichum*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Kregervanrija fluxuum*, and *Penicillium digitatum*. A mycobiome analysis of the tangerine peels using next-generation sequencing of internal transcribed spacer 1 region of fungal ribosomal RNA gene amplicon found that *P. digitatum* accounted for 98.4% of the fungus-derived gene sequences. However, precipitating antibodies against various fungi, including three *Penicillium* spp. (*P. digitatum*, *P. luteum*, *P. glabrum*), six *Aspergillus* spp., *Trichosporon cutaneum*, and *Aureobasidium pullulans*, were all negative in the serum of the patient.

The storehouse was extensively cleaned, and the patient worked there while wearing a mask. During the next winter and spring, there was no symptomatic recurrence, and the radiographic abnormalities of the lungs except for traction bronchiectasis gradually disappeared.

Discussion

There have been a few cases of OHP in citrus farmers reported to date, exclusively from Japan (12-14). Tangerines are harvested in late fall and stocked from December to February in storehouses, which was when and where the previous patients developed OHP (Table), whereas the cur-

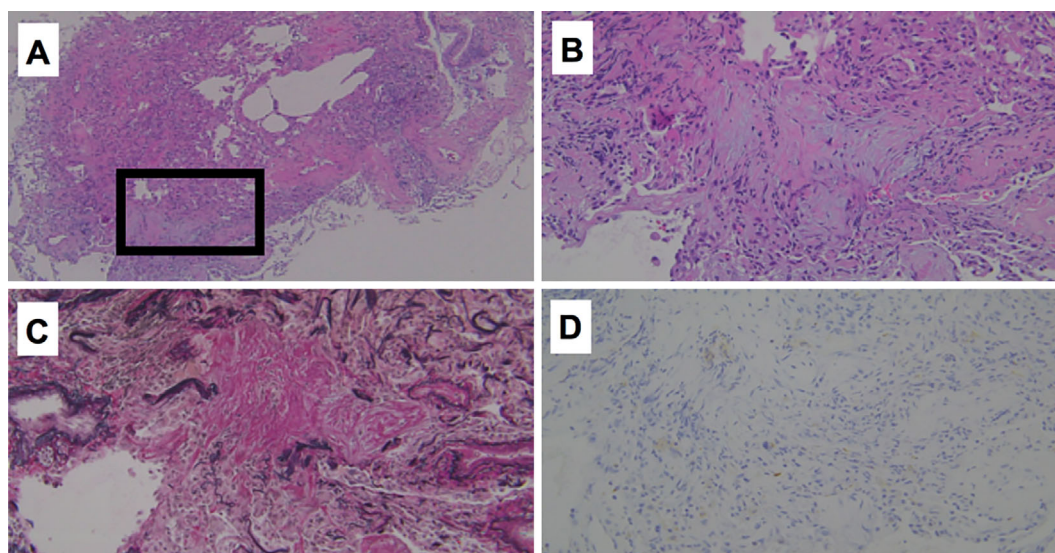


Figure 2. Pathological findings of a transbronchial lung biopsy sample. Hematoxylin and Eosin staining (A: $\times 40$, B: $\times 200$), Elastica van Gieson staining (C: $\times 200$), and immunohistochemistry of CD68 (D: $\times 200$).

Table. Characteristics and Laboratory Findings of Hypersensitivity Pneumonitis in Citrus Farmers.

Age/gender	50 F	67 F	53 M	62 F	66 M
Reference	12	12	12	13	
Symptoms	fever, cough, dyspnea	dyspnea, appetite loss	fever, cough, dyspnea	fever, cough	fever, dyspnea, fatigue
Admission	January	February	January	February	March
Medical histories	-	tuberculosis	-	dyslipidemia ectopic pregnancy	-
Tobacco	never	never	never	never	30 pack-years
Laboratory data					
WBC (μL)	15,120	4,520	8,060	7,800	12,200
Neutrophils (%)	(95.3)	(60.0)	(82.1)	(78.2)	(84.9)
LDH (IU/mL)	251	412	195	278	185
CRP (mg/dL)	0.1	0.2	6.8	9.08	14.83
KL-6 (IU/mL)	1,100	10,400	400	336	154
Bronchoalveolar lavage fluid					
TCC (mL)	3.05×10^5	1.9×10^5	1.92×10^5	6.3×10^5	4.47×10^5
Lymphocytes (%)	37	83.5	40.7	59	21
CD4/CD8	0.2	0.63	1.54	1.64	4.55
Serum precipitation antibody	<i>Penicillium luctem</i> <i>Aspergillus fumigatus</i> <i>Candida albicans</i> <i>Alternaria kikuchiana</i>	negative	<i>Aspergillus fumigatus</i>	<i>Candida albicans</i> <i>Penicillium luctem</i> <i>Cladosporium</i>	negative
Environmental culture	<i>Penicillium digitatum</i> <i>Aspergillus niger</i> <i>Cladosporium</i> <i>cladosporioides</i>	<i>Penicillium</i> sp.	<i>Penicillium</i> sp. <i>Mucor</i>	<i>Aspergillus niger</i> <i>Paecilomyces</i> sp.	<i>Penicillium digitatum</i>

CRP: C-reactive protein, KL-6: Krebs von den Lungen-6, LDH: lactate dehydrogenase, TCC: total cell count, WBC: white blood cell

rent patient grew a different variety of citrus harvested in late winter and developed symptoms in March. Peripheral blood leukocytosis was common, but the serum levels of CRP, Krebs von den Lungen-6, and lactate dehydrogenase were variable among cases. The modest lymphocytosis in the BALF and a high CD4/CD8 ratio in the present case may have been related to the fibrotic changes observed ra-

diographically and pathologically due to a longer and more insidious exposure to the antigen. CD4-positive lymphocytes are increased in the fibrotic lungs (15, 16) and localized in the fibrotic alveoli and lymphoid follicles, whereas CD8-positive T lymphocytes distribute diffusely in relatively normal alveoli (17).

We performed fungal culture and a mycobiome analysis

of the molded tangerine in the storehouse, finding that *P. digitatum* was the dominant fungus. *P. digitatum* and other *Penicillium* spp. were also the major air-borne fungi in the tangerine storehouses in other cases of citrus farmer's OHP (12, 13). However, we and other researchers failed to detect precipitating antibody to *P. digitatum* in the serum of the patients with citrus farmer's OHP, suggesting that 1) other pathogens had caused the disease; 2) this type of OHP is dominantly caused by different pathogenic mechanisms, such as Type IV hypersensitivity reactions (18); or 3) the tests using crude fungal extracts lacked sufficient sensitivity for the diagnosis of chronic HP. In fact, the sensitivity of serum IgG and IgA for recurrent and insidious types of chronic bird-related HP was only 48-61%, in contrast to 85-91% in cases of acute or recurrent-type disease (19). Further analyses are necessary to identify the causal antigen for tangerine worker's OHP.

Tangerine farms are mostly maintained as a family business in Japan, making it difficult for patients with OHP to avoid antigens by job change. It is, therefore, necessary to invent procedures that can reduce the amount of airborne antigen content and exposure in the workplace environment.

The authors state that they have no Conflict of Interest (COI).

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