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(Citation)
Respirology Case Reports,11(6):e01169

(Issue Date)
2023-06

(Resource Type)
journal article

(Version)
Version of Record

Received: 12 April 2023 | Accepted: 11 May 2023

DOI: 10.1002/rcr2.1169

CASE REPORT

Respirology Case Reports | WILEY

A case of eosinophilic pneumonia induced by lettuce

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Associate Editor: Uma Maheshwari Krishnaswamy

Abstract

A 56-year-old female lettuce farmer was admitted to the hospital with a low-grade fever, worsening cough, and dyspnoea. A blood test revealed eosinophilia and a high serum IgE concentration. The 3-year follow-up showed that her total IgE level increased in December, peaked in May, and suddenly decreased in August. This result was consistent with the lettuce harvest season. A chest x-ray taken on admission showed an infiltrative shadow in the upper lung field. Chest CT revealed patchy ground glass opacity on the upper lung field and thickening of the bronchial wall. The bronchoalveolar lavage fluid contained 8% eosinophils. She was treated with prednisolone, and her symptoms and radiological findings improved. The 37 kDa protein that reacted with the patient's sera was identified by immunoblot analysis.

KEYWORDS
allergy, eosinophilic pneumonia, lettuce

INTRODUCTION

Lettuce (*Lactuca sativa*) is a widely produced and consumed vegetable worldwide. Several studies have reported on the allergenicity of lettuce in relation to oral allergy syndrome, anaphylaxis, and occupational dermatitis. We reported that some lettuce farmer had respiratory symptoms during harvesting and packaging lettuce and identified epidermis-specific secreted glycoprotein EP1-like (51 kDa) as a new lettuce allergen.[1] Herein, we report the first case of eosinophilic pneumonia induced by lettuce in a lettuce farmer.

CASE REPORT

A 56-year-old female lettuce farmer was admitted to our department in May with a 2-week complaint of low-grade fever, worsening cough, and dyspnoea on exertion. She experienced the same episode in May ever year. And this was the first time she visited the hospital for these symptoms. She was treated with 200 mg of amikacin and 500 mg of azithromycin by a family physician, but the therapy had no effect on her low-grade fever, cough, or dyspnoea on exertion. The patient had been followed for thyroid gland polyp. She had no history of smoking, alcohol consumption, animal breeding, bird breeding, or chemical agent exposure. She had negative findings on her family history. She had not experienced episodic wheezing or rhonchi. She had been working as a lettuce farmer for 36 years. The lettuce harvest time is from December to June, with the largest harvest in April.

Her body temperature was 37.9°C, and her percutaneous oxygen saturation was 91% under ambient air conditions. Clubbing was not noted on her fingers. Physical examination of the head, neck, and abdomen was unremarkable. However, auscultation of her chest revealed bilateral wheezing during inspiration. Her arterial blood gas measurements under ambient air conditions were as follows: pH, 7.431; pO_2, 65.4 mmHg; pCO_2, 36.9 mmHg; HCO_3, 24.2 mmol/L.

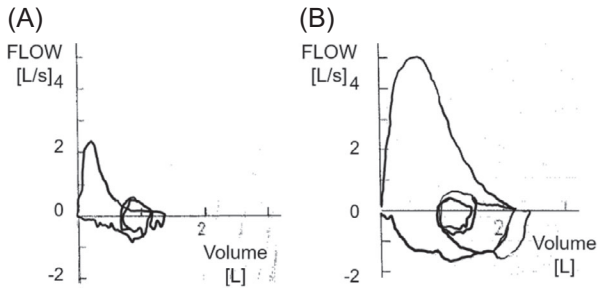
Her leukocyte count was 7330/μL. C-reactive protein was 3.55 md/dL.

Her chest x-ray revealed ground-grass opacity in the right upper lobe (Figure 1A). Plain computed tomography (CT) revealed mild infiltration, ground grass opacities, and reticulo-nodular shadows in both of the upper lobes, indicating interstitial pneumonia (Figure 1B).

Pulmonary function test (PFT) revealed mixed disorder of both obstructive and restrictive type (Figure 2A), although PFT revealed normal finding during non-harvesting season (Figure 2B). Intriguingly, the level of fractional exhaled nitric oxide (FeNO) was 42 ppb, suggesting that eosinophilic inflammation was present in airway. Skin prick test was positive for lettuce. Specific IgE test showed multiple allergies for timothy grass, orchard grass, cedar pollen, hinoki cypress pollen, Japanese white birch, cat, wheat, soybeans, peanut, latex, sesame, crab, kiwi fruit, peach, and tomato, although specific IgE test for lettuce is not commercially available.

Bronchoalveolar lavage fluid (BALF) obtained from the right S^3 (40/100 mL) showed elevated total cell counts (19.7×10^2/μL), with an increased percentage of eosinophils (8.5%). The ratio of CD4/CD8 lymphocytes was not detected because the percentage of lymphocytes was decreased to 1%. The BALF culture was negative for both bacteria and fungi.

The laboratory test results are shown in Table 1. The patient's Krebs von den Lungen-6 (KL-6) level was 226 U/mL (normal range < 500 U/mL). Her serum total immunoglobulin-E (IgE) level was greatly increased (3038.9 IU/mL), and the D-glucan value was decreased to <11 pg/mL. After admission to our hospital, her clinical symptoms gradually improved within a week with 0.5 mg/kg prednisolone (PSL). Even after a gradual decrease in the PSL, her dyspnoea did not flare up. However, she had general malaise and a mild dry cough during the lettuce harvesting season without abnormal image findings. The 3-year follow-up showed that her total IgE level increased in December, peaked in May, and suddenly decreased in August. This result was consistent with the lettuce harvest season. The leaf harvest period started in December, peaked in May, and ended in June. The written informed consent for the publication was obtained from the patient.



VC (L)	1.59	VC (L)	2.29
%VC (%)	61.2	%VC (%)	88.8
FVC (L)	1.47	FVC (L)	2.29
FEV_1 (L)	0.79	FEV_1 (L)	1.81
FEV_1/FVC (%)	53.74	FEV_1/FVC (%)	79.04
%FEV_1 (%)	36.2	%FEV_1 (%)	83.4

FIGURE 2 (A) Pulmonary function test (PFT) showing mixed disorder during lettuce harvesting seasons. (B) PFT showing normal finding during non-harvesting season.

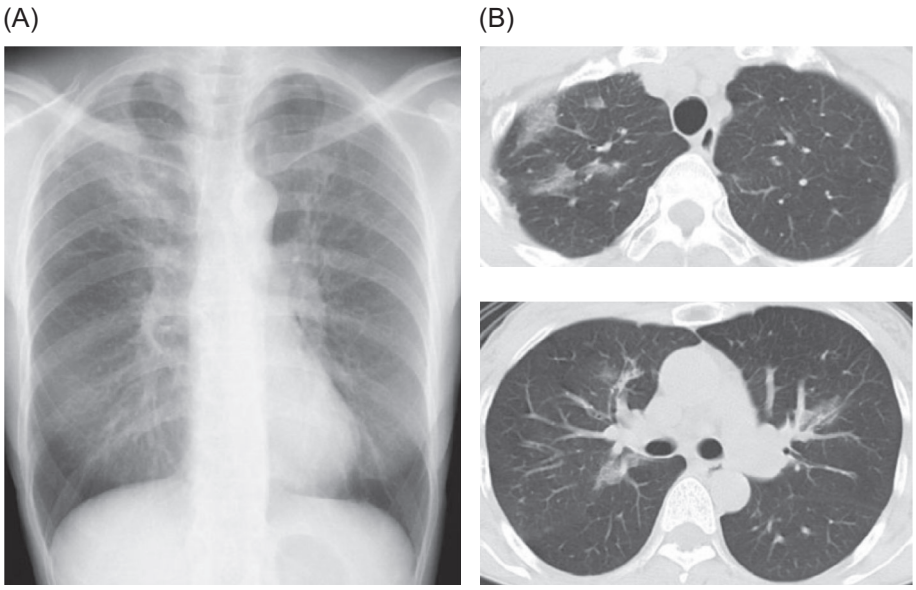


FIGURE 1 (A) Chest x-ray showing ground grass opacity in the right upper lobe. (B) Chest plain CT showing mild infiltration, ground grass opacities, and reticulo-nodular patterns in both of the upper lobes, indicating interstitial pneumonia.

TABLE 1 Laboratory data on admission.

Peripheral blood				**Immunoserology**			
WBC	7.33×10^3	$4.2–10.0 \times 10^3$	/μL	ANA	40	<80	
Neutrophil	5.17×10^3		/μL	PR3-ANCA	<1.0	<1.0	
	70.5	40–74	%	MPO-ANCA	<1.0	<1.0	
Lymphocyte	0.95×10^3		/μL				
	13	19–48	%				
Eosinophil	0.72×10^3		/μL				
	9.8	0–7	%				
Monocyte	0.32×10^3		/μL				
	4.4	3.4–9	%				
Basophil	0.12×10^3		/μL	**Blood gas (4 L O_2)**			
	1.7	0–1.5	%				
Haemoglobin	14.4	12–15	g/dL	pH	7.431	7.35–7.45	
Ht	40.9	35–48	%	$PaCO_2$	36.9	32–45	Torr
Plt	26.5	14.8–35.8	/μL	PaO_2	65.4	83–108	Torr
				HCO_3^-	24.2	21–28	mmol/L
Blood chemistry							
Total Bil	0.46	0.1–0.8	g/dL				
AST	23	10–34	IU/L	**BALF**			
ALT	16	6–37	IU/L	Total cell count	19.7×10^2		/μL
LDH	275	106–211	IU/L	Cell differentials			
BUN	13.4	8.5–20	mg/dL	Macrophage	84		%
Cr	0.51	0.43–0.72	mg/dL	Lymphocyte	1		%
Na	135	135–147	mmol/L	Eosinophil	8.5		%
K	3.9	3.6–5.0	mmol/L				
Cl	100	96–107	mmol/L				
CK	68	29–135	IU/L	**BALF culture**			
Glu	113	70–110	mg/dL	Negative			
CRP	3.55	0–0.6	mg/dL				
KL-6	226	<500	IU/mL				

IMMUNOBLOT ANALYSIS

A fresh lettuce center core was cut using a sharp scalpel, and the white juice was spread on a section and collected. This lettuce center core juice was dissolved in distilled water to prevent hardening (i.e., 20 μL of white juice in 500 μL of distilled water).

The lettuce center core juice protein (approximately 15 μg of protein) was separated through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins on the gel were stained with Coomassie Brilliant Blue R-350 (GE Healthcare, Chicago, USA) to detect the total protein patterns (Figure 3A).

Immunoblot analysis was conducted by transferring the SDS-PAGE gel onto an Immobilon-P™ PVDF membrane (Merck Millipore, Burlington, MA) by using a semi-dry blotting method.[2] The membrane was incubated in 10 mM PBS-T (pH 7.5) and 5% skim milk for blocking. The membrane was then incubated overnight at 4°C in diluted serum (20-fold) in the same blocking buffer. After washing the membranes 4 times with PBS-T for 10 min, the bound primary antibodies were detected by using 5000-fold HRP-conjugated goat anti-human IgG mouse-monoclonal antibody (Jackson Immunoesearch Laboratory, West Grove, PA) and an ECL western blotting kit (GE Healthcare, Boston, MA). After washing the membranes 4 times with PBS-T for 10 min, the resultant chemiluminescent signals were detected on X-ray film (Hyperfilm MP, GE Healthcare). The sera from non-atopic healthy volunteers were used as negative controls.

Figure 3B shows the binding band of the specific IgG from the sera of the patient and a non-atopic healthy volunteer. The 37 kDa band was recognized in only the patient's serum. Control immunoblot assays with sera from non-atopic patients did not show any IgG-binding bands.

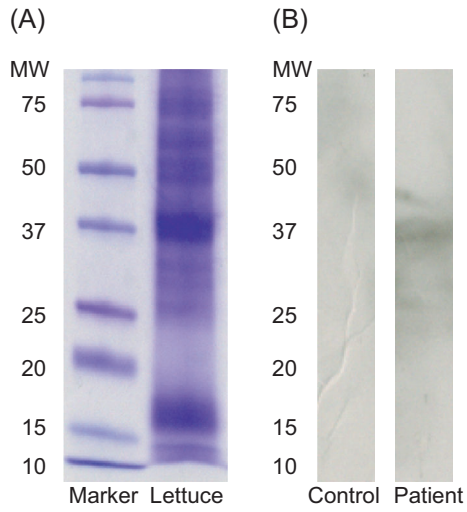


FIGURE 3 (A) Coomassie Brilliant Blue-stained SDS-PAGE gel of the lettuce centre core juice. Marker and molecular weights (MW, kDa) are indicated on the left. (B) IgG-binding band of the lettuce centre core juice by immunoblotting is shown. Lane Patient represents the serum from the patient and contains a single protein band at 37 kDa. Lanes Control represents the negative control sera from non-atopic volunteers. MW, molecular weight marker (kDa).

DISCUSSION

The following criteria are considered to be significant for the diagnosis of hypersensitivity pneumonitis: respiratory compatible symptoms (dry cough, dyspnoea, and bilateral fine crackles), presence of alveolar eosinophilia in the BAL fluid, and radiologic elements (ground-glass opacities and linear atelectasis).[3] However, BAL lymphocytosis was not present in this case. The diagnosis of acute eosinophilic pneumonia was not applicable to this case because the patient presented 25% > eosinophils. But the absence of lymphocyte in BALF makes it hard to diagnosis as hypersensitivity pneumonitis, because hypersensitivity pneumonitis generally reveals diffuse distribution in the lung. On the other hand, eosinophilic pneumonia may have different BAL findings from place to place. In addition, eosinophilia in blood, increased eosinophils in BALF, elevated FeNO level and increased total IgE suggests that patient are matched with eosinophilic pneumonia probably complicated with asthma induced by lettuce, rather than hypersensitivity pneumonitis. The lack of specific provocation test and biopsy were limitations of this case report.

SDS-PAGE and IgG immunoblotting showed that the patient's serum contained lettuce-specific IgGs compared to the negative control sera. The specific IgG-binding bands were identified in the white lettuce juice from the center core at approximately 37 kDa. Though no case of lettuce-induced HP has been reported in the past, a Japanese case report concerning asthma among lettuce farmers has been published.[4] In cases of respiratory symptoms in mushroom workers, the spores from the mushrooms induced pollinosis, rhinitis, asthma and HP.[3,5,6] Similar to mushroom-related respiratory symptoms, it is suggested that there is a gradation of symptoms in lettuce-related respiratory allergies, from asthma to HP. The current patient had acute respiratory symptoms during harvesting season and did not have symptoms during non-harvesting season. Therefore, the avoidance from antigen is quite useful in this patient. Although complete avoidance from antigens is very important, it is difficult to stop lettuce cultivation for economic reasons in some cases.

In conclusion, we demonstrated a case of lettuce-associating pneumonitis for the first time. For this patient, avoidance of allergens was difficult because lettuce farming was her family's main source of income. Wearing a mask and reducing the amount of the crop improved her symptoms the following year.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

We will submit upon request.

ETHICS STATEMENT

The authors declare that appropriate written informed consent was obtained for the publication of this manuscript and accompanying images.

DISCLOSURE

A version of this manuscript is available on the preprint server: https://www.researchsquare.com/article/rs-1029130/v1.

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How to cite this article: Sekiya R, Nagano T, Moriyama T, Kawaguchi A, Fukui T, Mimura C, et al. A case of eosinophilic pneumonia induced by lettuce. Respirology Case Reports. 2023;11:e01169. https://doi.org/10.1002/rcr2.1169