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Case report

Humidifier lung induced by endotoxin and various pathogens: Characteristic differences from other phenotypes of hypersensitivity pneumonitis

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ABSTRACT

A 46-year-old man visited our hospital with a fever and cough. The symptoms had started two months after continued use of an ultrasonic humidifier. He had hypoxemia on admission and late inspiratory crackles in both lungs on physical examination. The laboratory findings showed an increased white blood cell count and a C-reactive protein level, and his serum KL-6 level was slightly elevated, at 674 U/mL. Chest computed tomography showed diffuse ground-glass opacities, and histological examination of a transbronchial lung biopsy showed alveolitis without granulomas. The humidifier inhalation challenge test result was positive. Therefore, we diagnosed the patient with humidifier lung. His symptoms gradually improved after avoiding the humidifier without taking medication. The humidifier water was contaminated by various bacteria and fungi, as well as *Mycobacterium gordonae* and a high concentration of endotoxin. Unlike in those with typical hypersensitivity pneumonitis, the elevation of serum KL-6 levels in humidifier lung patients is mild, and granulomas are not apparent on histological examination, similar to our case. Furthermore, the endotoxin identified from the humidifier is one of the known pathogens of humidifier lung. Thus, humidifier lung seems to have different characteristics compared to other hypersensitivity pneumonitis phenotypes. The mechanism driven by the high concentration of endotoxin could be one of the main causes of humidifier lung.

1. Background

Humidifier lung is a rare phenotype of hypersensitivity pneumonitis (HP) caused by inhalation exposure to contaminated ventilation units, such as a humidifier [1]. In past reports, various pathogens were reported as causative agents, such as *Thermoactinomyces*, fungi, and other bacteria [2]. Some case reports indicated that high levels of endotoxin in the contaminated water caused the disease without a specific antigen [2]. Here, we described a case in which a high concentration of endotoxin and several pathogens were identified in the humidifier water. Interestingly, we found that the features of humidifier lung were different from those of other phenotypes of HP.

2. Case presentation

A forty-six-year-old man suffered from a fever and nonproductive cough for 2 weeks. He noticed that his symptoms started two months after he started running an ultrasonic humidifier 24 hours a day in his home. He went to his local doctor and was prescribed medicine for bronchitis containing clarithromycin and amoxicillin. However, his symptoms worsened, and he visited our hospital in the winter, two weeks after his first visit. The patient, who was a smoker (10 pack-years), had a medical history of depression and sleep apnea syndrome, without medical treatment. His occupation was an automotive maintenance engineer.

His vital signs on admission showed a low grade fever of 37.0°, moderate tachycardia of 98 beats per minute, tachypnea of 20 times/min, and hypoxemia with an oxygen saturation level of 88% on ambient air. Physical examination revealed no abnormalities except for inspiratory late crackles in both lung bases. An arterial blood sample showed a $PaCO_2$ of 36.9 mmHg and a PaO_2 of 59.8 mmHg. The laboratory findings showed an increased white blood cell count of 10700/μL with 72.8% polymorphic nuclear leukocytes and a C-reactive protein (CRP) concentration of 7.24 mg/dL. His serum levels of KL-6 (674 U/mL) and SP-D (151 ng/mL) were slightly elevated. The serum antibodies against

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Abbreviation

HP	hypersensitivity pneumonitis
WBC	white blood cell count
CRP	C-reactive protein
CT	computed tomography
DL_{CO}	% single breath carbon monoxide diffusing capacity
BALF	bronchoalveolar lavage fluid
CD4/8	the ratio of $CD4^+$ to $CD8^+$ cells
M. gordonae	Mycobacterium gordonae
T. vulgaris	Thermoactinomyces vulgaris
T. sacchari	Thermoactinomyces sacchari
T. candidus	Thermoactinomyces candidus
ODTS	organic dust toxic syndrome
IQR	interquartile range
TBLB	transbronchial lung biopsy

Trichosporon asahii and *Aspergillus* were negative. Chest high-resolution computed tomography (CT) showed bilateral diffuse subpleural nonsegmental ground-glass opacities (Fig. 1). The pulmonary function test showed a restrictive pattern (predicted forced vital capacity 75.9%) with moderately reduced diffusion capacity (% single-breath carbon monoxide diffusing capacity 71.5%).

Based on the clinical course and the findings of the examinations, we suspected HP associated with the ultrasonic humidifier and performed flexible bronchoscopy to obtain a definitive diagnosis. The bronchoalveolar lavage fluid (BALF) revealed an elevated cell count (16.4×10^6/mL) with lymphocytes (51.0%), neutrophils (11.4%), and eosinophils (8%). The ratio of $CD4^+$ to $CD8^+$ cells (CD4/8) of BALF was 1.60, which was normal. The histological examination of the transbronchial lung biopsy (TBLB) showed alveolitis with the infiltration of lymphocytes and plasma cells without granulomas or Masson bodies. We performed an inhalation challenge test with the ultrasonic humidifier (Fig. 2). Six hours after the test, he developed a high fever (38.1° Celsius), chills and dyspnea. The blood tests showed an increased white blood cell count (29950/μL) and CRP level (4.85 mg/dL) on the following day, while the serum KL-6 level did not change. Accordingly,

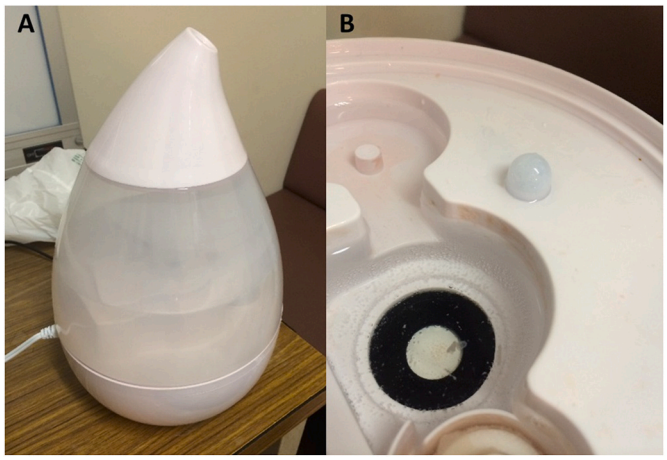

Fig. 2. The ultrasonic humidifier the patient used.
A: The patient's ultrasonic humidifier.
B: Humidifier water in the tank. There was white and pink residue adhered to the tank. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

we diagnosed the patient with humidifier lung. His symptoms, laboratory findings, and radiographic findings gradually improved after avoiding the use of the humidifier without taking medication.

Acinetobacter junii, *Bacillus spp*, *Cladosporium cladosporioides*, *Fusarium graminearum*, and *Mycobacterium gordonae* were isolated from the humidifier water, while no pathogens except for normal flora were isolated from the BALF. The concentration of endotoxin in the humidifier water was high (more than 5000 pg/dL) (Table 1).

3. Discussion

Humidifier lung is a rare phenotype of HP (4.3%) in Japan [3]. In the present case, we found some unique clinicopathological findings compared with those of other phenotypes of HP. The ultrasonic humidifier was contaminated with various pathogens, such as gram-negative rod bacteria and fungi, and we also identified a high concentration of endotoxin. Moreover, the elevation of the serum KL-6

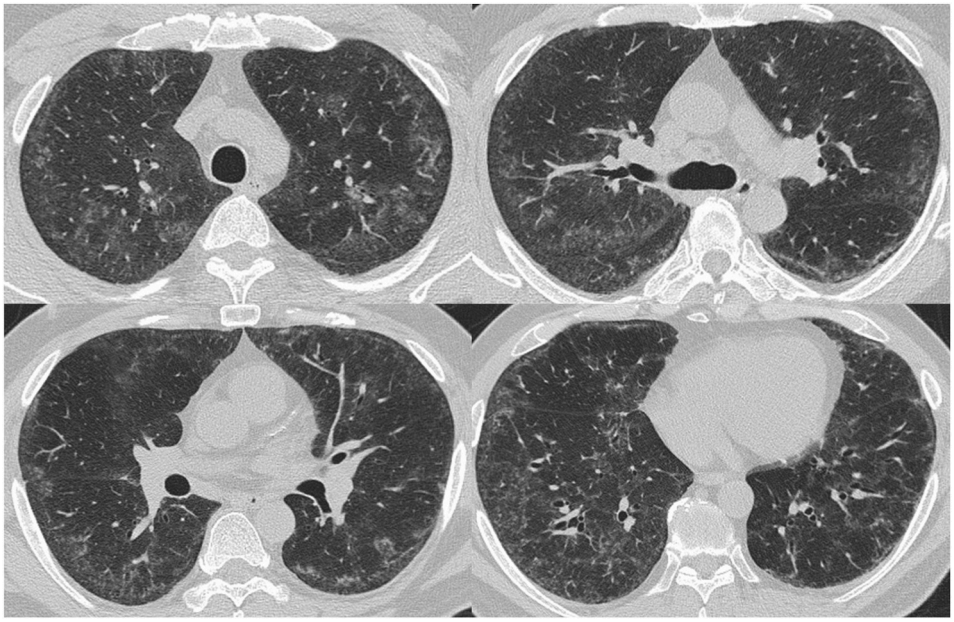

Fig. 1. Chest high-resolution computed tomography (CT).
The chest CT showed diffuse subpleural nonsegmental ground-glass opacities.

Table. 1
The laboratory findings

Hematology		Biochemistry		Arterial blood gas	
Hb	12.5 g/dL	Na	138 mmol/L	pH	7.419
Ht	36.7%	K	4.3 mmol/L	PaCO2	36.9 torr
RBC	429x104 /μL	Cl	104 mmol/L	PaO2	59.8 torr
WBC	10700 /μL	BUN	17.0 mg/dL	HCO3	23.3 mmol/L
Seg	72.8%	Cr	0.93 mg/dL	BE	-0.8 mmol/L
Eo	4.5%	TP	6.68 g/dL	**Pulmonary function test**	
Baso	0.4%	Alb	3.75 g/dL	%VC	75.9%
Mono	6.4%	Glb	2.93 g/dL	FEV1%	90.1%
Plt	38.7x104 /μL	T-bil	0.7 mg/dL	**Bronchoalveolar lavage**	
		AST	13 IU/L	Total cell count	16.4x106 /mL
		ALT	7 IU/L	Macrophages	29.6%
		LDH	160 IU/L	Lymphocytes	51.0%
		CK	75 IU/L	Neutrophils	11.4%
		CRP	7.24 mg/dL	Eosinophils	8.0%
		KL-6	674 U/mL	CD4/8 ratio	1.6
		SP-D	151 ng/mL	Laboratory findings of Humidifier water	
		(1→3)-β-D-glucan	<6.0 ng/mL	(1→3)-β-D-glucan	>2151 pg/mL
				Endotoxin	>5000 pg/dL

level was mild, and granulomas did not appear in the histological examination of the TBLB. We suspect that these findings indicate that a toxic reaction might be a primary contributor to the pathogenesis of humidifier lung in addition to an allergic reaction.

Various fungi and/or bacteria have been reported as causative agents of humidifier lung [4]. Thermophilic actinomycetes (*T. vulgaris*, *T. sacchari*, and *T. candidus*) are the pathogens most frequently isolated from evaporating or steam humidifiers [5,6]. However, recent reports have described that different pathogens are isolated from ultrasonic humidifier water, which has a lower temperature than evaporative or steam humidifiers. Table 2 shows the causative antigens and the respective phenotypes [1,2,5–7]. In the present case, *C. cladosporioides, F. graminearum*, and *M. gordonae* could have been the causative agent; we did not measure each antibody.

Generally, species-specific antibodies should be evidence of sensitization in HP patients [6]. However, among 17 past case reports that examined precipitating antibodies, only eleven patients (64.7%) were identified as having precipitating antibodies against pathogens identified in humidifier water [2,8]. Furthermore, endotoxin identified from humidifiers constitutes one of the known inducers of humidifier lung [2,4,8]. A high concentration of endotoxin in the humidifier water was reported in a case in which the patient had humidifier lung but was negative for serum-precipitating antibodies [8].

Table 2
Etiologic agents of each phenotype of hypersensitivity pneumonitis.

Phenotype of HP[a]		Antigen
Summer type		*Trichosporon asahii*
Bird fancier's lung		Avian droppings, feathers, serum
Farmer's lung		*Saccharopolyspora rectivirgula* (*Micropolyspora faeni*), *Aspergillus* spp, *Thermoactinomyces vulgaris*, *Thermoactino myces viridis*, *Thermoactinomyces sacchari*, *Fusarium spp*, *Candida spp*, *Penicillium spp*, and animal fur protein
Humidifier lung	Steam	Thermophilic actinomycetes (*Thermoactinomyses vulgaris*, *Thermoactinomyses candidus*), *Micropolyspora faeni*
	Ultrasonic	Fungi: *Cephalosporium* spp, *Trichosporon viride*, *Trichoderma* spp, *Candida* spp, *Aspergillus fumigatus*, *Cladosporium* spp, *Pullularia pullulans*, *Trichoderma viride, Fusarium* spp Bacteria: *Klebsiella oxytoca*, aerobic gram-positive bacilli, Acid-fast bacillus: *Mycobacterium gordonae* Other: endotoxin, Amebae

[a] Hypersensitivity pneumonitis.

Interestingly, *A. junii*, *Bacillus* spp., and a high concentration of endotoxin were also identified in the present case. Genma H et al. reported that gram-negative pathogens are broken down by ultrasonic humidifiers [9], and the generated bacterial endotoxin is associated with lung injuries such as organic dust toxic syndrome (ODTS) [8]. Endotoxin exposure causes neutrophilic inflammation through the release of the complement cascade component C5a and interleukin-8 from alveolar macrophages and bronchial epithelial cells [10,11]. The underlying mechanisms are different from those in other HPs, showing type III and IV allergic reactions.

In this case, the patient's serum KL-6 levels were not much elevated. Serum KL-6 is produced on alveolar type II pneumocytes, and the level increases due to regenerating alveolar type II pneumocytes and/or enhanced permeability following the destruction of the air-blood barrier [12]. Generally, serum KL-6 levels in patients with HP are highly elevated median (interquartile range (IQR): 2700 U/mL (1510–5710 U/ml)), and they are significantly higher than those in patients with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia [12]. However, the serum KL-6 levels in our patient and in previous reports of humidifier lung were within the normal range or were only slightly increased [4,8]. Moreover, the pathological findings showed alveolitis without granulomas. In a previous report, epithelioid cell granulomas were less commonly detected in humidifier lung patients than in those with other phenotypes of HP [2]. Those findings regarding humidifier lung might resemble acute respiratory distress syndrome associated with inhalation exposure to a spray, with alveolitis as the characteristic histopathological findings, unlike in other phenotypes of HP [13,14]. Our patient was a current smoker. Past reports have shown that nicotine suppresses macrophage activation and lymphocyte proliferation and function; therefore, cigarette smoking is thought to exert a protective effect against the development of HP [1]. However, this protective effect has not been shown in patients with humidifier lung.

From the above evidence, it should be considered whether the exposure to a high concentration of endotoxin could be the more important mechanism underlying humidifier lung than type III and IV allergic reactions, which lead to a weaker reaction of type II pneumocytes and the formation of granulomas. To test this hypothesis, it is necessary to review the cases of humidifier lung and the other phenotypes of HP on a large scale as well as to examine the vapor of the humidifier water and perform immunological analyses as fundamental research to clarify the interactions of the pathogens and endotoxin with

the host. However, these pathological findings cannot deny the presence of granulomas because of the limitations of TBLB.

4. Conclusion

This reported case of humidifier lung had different features from those of other phenotypes of HP, such as a mild elevation of the serum KL-6 level, no granulomas observed on histological examination, and highly concentrated endotoxin in the humidifier water. The presence of these unusual findings could indicate this phenotype. Therefore, the mechanism driven by the high concentration of endotoxin could be one of the main causes of humidifier lung. Additional case studies and review of the existing case studies are warranted to clarify our findings in the future.

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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None.

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