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CASE STUDY

Hypersensitivity pneumonitis caused by occupational exposure to phytase

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ABSTRACT: A 43-yr-old male presented with a 6-month history of episodes of coughing, shortness of breath and fever. He suffered from dyspnoea on minor exertion. The patient worked in a cattle feed factory and noticed that he had more complaints after his working hours. His symptoms could be ascribed to hypersensitivity pneumonitis due to contact with phytase, an enzyme added to cattle feed to strengthen bone and diminish phosphorus excretion.

The diagnosis was supported by bibasal lung crackles on physical examination, restrictive ventilatory defect (with decreased diffusion capacity for carbon monoxide), typical radiographical findings, lymphocytosis in bronchoalveolar lavage fluid and a positive exposure test performed at the workplace. Blood examination showed high immunoglobulin G levels to phytase.

After treatment and cessation of phytase contact the patient became symptom free and lung function normalised. Phytase should be considered as a cause of occupational hypersensitivity pneumonitis in the animal feed industry.

KEYWORDS: Extrinsic allergic alveolitis, hypersensitivity pneumonitis, phytase

CASE REPORT

A 43-yr-old male without prior illness was referred to the outpatient clinic of our department with symptoms of cough and shortness of breath on minor exertion, fever and malaise (especially fatigue). He had lost 8 kg of weight since his complaints had started 6 months earlier and had been a non-smoker for 20 yrs. There was a relationship between his complaints and his working hours. He had worked in a factory that produces cattle feed for >20 yrs where his role was to take samples of the cattle feed at different departments on different floors for quality control. He noticed that after having taken samples on one particular floor, 6–8 h later, his complaints started. The next morning the complaints had almost disappeared. He had no symptoms during the holidays. None of his colleagues (n=21) had similar symptoms.

At physical examination pulse and blood pressure were normal. At auscultation of the lungs normal breath sounds were heard but with bibasal crackles. Erythrocyte sedimentation rate was 33 $mm \cdot h^{-1}$ and C-reactive protein was 11 $mg \cdot L^{-1}$ (normal range: <10 $mg \cdot L^{-1}$). Blood leukocytes, total immunoglobulin (Ig)E, antinuclear antibodies and specific IgE for common aeroallergens were all within reference values.

A chest radiograph showed increased ill-defined parenchymal opacities especially in the lower lung fields. The high-resolution computed tomography of the thorax showed a uniform distribution of ill-defined centrilobular nodules with mainly ground-glass pattern (fig. 1).

Lung function testing, predicted values according to the European Community for Coal and Steel [1], showed a mild restrictive pattern with a decreased diffusion capacity for carbon monoxide. The vital capacity (VC) was 4.6 L (82% predicted), total lung capacity (TLC) was 6.0 L (75% pred) and diffusing capacity of the lungs (D_L) was 6.2 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (51% pred). During exercise testing on a cyclometer the patients' maximum load was 180 W (77% pred) and oxygen tension decreased during exercise from 10.99 kPa to 7.19 kPa.

On the particular factory floor, aerosolised phytase (curtain of vaporised phytase) is added to the animal feed by a conveyor belt in a closed system. When taking the samples, the patient did not wear a respiratory protective device.

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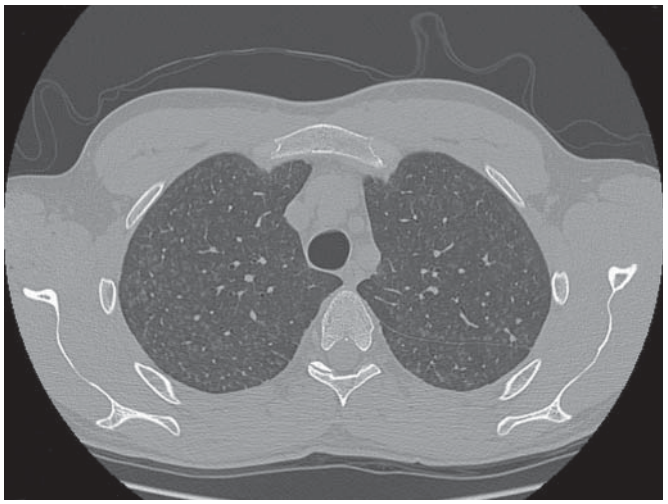

FIGURE 1. High-resolution computed tomography of the thorax obtained when the patient presented with symptoms. Ill-defined centrilobular nodules are visible.

Because his symptoms were related to his work with phytase, an exposure test was carried out at the workplace. During this test the patient took samples of pig's feed for 10 min from the area where the feed is carried through a curtain of vaporised phytase by a conveyor. He did not do any other work on that particular day.

He developed progressive dyspnoea on exertion, fever (up to 38.5°C) with malaise, nausea and vomiting 6 h after the exposure. The following morning bronchoalveolar lavage (BAL) was performed [2]. The total number of cells was elevated (40×10^6 cells·mL^{-1}), especially the number of neutrophils (37.6%) and lymphocytes (38%), with a decreased CD4/CD8 ratio of 0.53. The percentages of macrophages (21%) and of eosinophils (4%) were both elevated.

To demonstrate inhalable phytase concentrations in the air, especially on that particular floor, airborne inhalable dust was sampled on Teflon filters (10 filters, 2.8–7.6 h sampling time) on various floors of the factory with stationary Gilian pumps (Sensidyne, Clearwater, FL, USA) and PAS-6 sampling heads (IRAS, Utrecht, The Netherlands). The dust was weighed, extracted and measured with a sandwich enzyme immunoassay based on *Aspergillus niger* phytase-specific rabbit antibodies [3], but with phytase that was used in the factory and produced in *Trichoderma reesei* (Finase® L; Behn Meyer, Subang Jaya, Malaysia) as the standard. The *T. reesei* strain used for production of Finase® contained the gene for a 3-phytase from *A. awamori*. Using inhibition experiments with the rabbit antibodies, cross-reactivity between phytases from both Aspergillus species could be shown (data not shown). With Finase® L as a standard, the limit of detection was 1.3 ng·mL^{-1} (or 3.4 ng·m^{-3} for 8-h sampling). On the floor of the factory where Finase® L was added, the inhalable dust concentration was 0.5–1.1 mg·m^{-3} while the phytase concentration was 8.7–38.4 μg·m^{-3} (16.5–38.7 μg·mg^{-1}, n=8). On the other floors, phytase concentrations were much lower (6–58 ng·m^{-3}, 1–18 ng·mg^{-1}, n=2). IgG and IgE levels against different antigens [4] (table 1) were measured in the patient's serum by UniCap 100 (Phadia, Freiburg, Germany).

The patient was treated with steroids (prednisone, 30 mg·day^{-1}) for 1 week and with beclomethasone 200 μg twice daily by aerosol inhalation. The patient's symptoms decreased. While at work, he started to wear a protective mask (Arteli Virgo FFP3/v-EN149;2001-FFP3-CE-0086). A repeated chest radiograph and lung function test 2 months after treatment were normalised. The VC had improved to 5.8 L (103% pred), TLC was 7.6 L (90% pred) and D_L was 7.5 mol·min^{-1}·kPa^{-1} (62% pred).

DISCUSSION

The patient's medical history, bibasal lung crackles at auscultation, the restrictive lung function pattern with decreased diffusion capacity for carbon monoxide, oxygen tension decrease on exertion, radiological features, increased specific IgG to phytase, and the positive exposure test (including the BAL results), all support the diagnosis of hypersensitivity pneumonitis (HP) [5–7]. Furthermore, the clinical improvement after treatment and cessation of the contact to phytase strongly suggest HP due to inhalation of phytase. To the best of our knowledge, this has never been reported before.

Phytase is an enzyme that catalyses the hydrolysis of phytate, a phosphate-storage mode present in cereals and soy, to inorganic phosphate. Monogastric animals (pigs and poultry) can only partially utilise this form of phosphorus. To increase the bioavailability of phosphorus for these types of animals, fungal phytase is added to the feed. This results in a decreased need to add inorganic phosphorus and a reduction of faecal phosphorus excretion [8, 9].

Our data show a strong relationship between the contact with phytase and the patient's symptoms. Even though the exposure test was carried out in the workplace instead of a

TABLE 1 Immunoglobulin (Ig) levels

IgE against phytase from *Aspergillus niger*	Negative#
IgE against phytase from *Aspergillus awamori* expressed in *Trichoderma reesei*	Negative#
IgG against phytase from *Aspergillus awamori* expressed in *Trichoderma reesei*	2450 mgA·L^{-1}#
IgG against phytase from *Aspergillus niger*	722 mgA·L^{-1}#
IgG to *Trichoderma viride* (m15)	101 mgA·L^{-1}
IgG to mould mixture (mx1)	34 mgA·L^{-1}
IgG to *Aspergillus fumigatus* (gm3)	26 mgA·L^{-1}

#: biotinylated antigen/allergen bound to Streptavidin (ImmunoCAP; Phadia, Uppsala, Sweden) as described previously [4].

laboratory, the positive challenge test, exposure measurements from the factory and patient's high levels of specific IgG to *A. awamori*-phytase produced in *T. reesei* are strong arguments for the relationship between phytase and the disease.

While the patient had extremely high IgG levels against phytase products, he had only moderate IgG levels against *T. viride, A. fumigatus* and a mould mixture. Thus, it seems unlikely that HP was caused by ''mould'' or ''mould-contamination''.

The positive effect of wearing a respiratory device as a protective measure in HP hasn't been proven [10]. In occupational asthma wearing a respiratory device can reduce respiratory symptoms but they don't offer complete protection [11, 12]. In our case, the patient hasn't demonstrated any respiratory symptoms following treatment and since wearing a protective mask.

No previous studies or case reports have described a relationship between HP and phytase or another phosphohydrolase. However, there are a few studies that described phytase as the cause for (allergic) respiratory symptoms. The reason for this might be that phytase as an additive has been used only since the early 1990s. DOEKES *et al.* [13] described that exposure to phytase from *A. niger* can cause IgE sensitisation, occupational asthma and other respiratory symptoms. O'CONNOR *et al.* [14] have proved by specific bronchial challenge that phytase can cause occupational asthma. In addition, BAUR *et al.* [9] confirmed that powdered phytase derived from *A. niger* is a highly sensitising substance and, recently, CABALLERO *et al.* [15] showed that phytases from Peniophora and Trichoderma species caused IgE-mediated allergy in animal feed industry workers. In the present case, the sensitising phytase was an *A. awamori* 3-phytase expressed in *T. reesei*. This enzyme was detected in the workplace by rabbit antibodies developed against *A. niger* phytase. As the amino acid sequences of these enzymes are >97% identical, it is no wonder these antibodies and, additionally, the patient's IgG reacted to phytases from both species. Airborne phytase concentrations at the patient's workplace reached 38.4 $\mu g \cdot m^{-3}$ which is much higher than, for example, protease concentrations in the detergent industry [16]. This exposure caused severe symptoms in the evening after taking samples for only 10 min, and high-antigen exposure may also be an important step in the pathogenesis of the patient's disease. HP is mostly caused by a highly contaminated area of a causative agent. In our case, the working area contained high levels of the causative agent in contrast to other places within the factory.

In contrast to the cases that have been described in literature, our patient did not develop an immunoglobulin E mediated allergy to phytase but hypersensitivity pneumonitis.

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