

Experimental method for isolating and identifying dust mites from sputum in pulmonary acariasis

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Abstract The aim of this study was to pilot a simpler and more effective method for identifying dust mites in sputum. Dust mites and their allergens have been implicated in respiratory diseases, including pulmonary acariasis, and several studies have identified mites in sputum. Further research is dependent on the development of a faster and simpler diagnostic test. We have demonstrated that dust mites artificially introduced into sputa, could be identified after the sputa were liquefied with bleach, when the liquid sample was observed under the microscope. Liquefaction times for serous, mucous, purulent and hemoptoic sputa varied from 10 to 30 min (mean 17.5). The test had a sensitivity of 80% (95% CI 68.2–88.2%) as 46/60 mites were identified. This procedure can be performed quickly at room temperature, is simple, inexpensive, repeatable, and less labourious than previous methods.

Keywords Dust mites · *Dermatophagoides* · Sputum liquefaction · Bleach · Diagnostic test

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Introduction

There are a number of references in the published literature to the presence of dust mites in sputum (Carter et al. 1944; Farley et al. 1989; Ryu et al. 2003) and the inhalation of dust mites has been postulated (Nadchatram 2005). The term pulmonary acariasis has sometimes been used. The presence of mites in sputum merits further research, which has been inhibited by the lack of a suitable diagnostic test. Consequently, our aim in this study has been to develop a simple and effective method for identifying dust mites in sputum. This test could then be used to identify the presence and number of mites in the sputum of patients with respiratory symptoms, and determine the prevalence of pulmonary acariasis in a population of patients with respiratory symptoms.

The characteristics of sputum, in particular its consistency, mean that liquefaction and homogenisation are required prior to examination of a sample. Various methods, both physical and chemical, have been employed to this end (Grebski et al. 2001). Furthermore, the use of solutions of sodium hypochlorite to liquefy samples from the respiratory tract (sputum and bronchoalveolar lavage) have been documented for some time in relation to the finding of amosite asbestos and ferruginous bodies (Paris et al. 2002; Roggli et al. 1986).

Various procedures have been used to extract dust mites from different types of substrata. The most widely used method is flotation, which is based on the difference in specific gravity that exists between dust mites and various liquid media (Thind 2000; Proctor 2001). These methods allow quantitative determination of the number of mites present and identification of the specific mite species. To a greater or lesser degree, these procedures are labour intensive, time consuming, and poorly suited to the examination of sputum.

The immediate objective of the study was to determine whether it was possible to identify dust mites that had been artificially introduced into sputum, by observation of the liquid sample under the microscope, after liquefaction of the sample with bleach.

Materials and methods

An initial experiment was undertaken to determine the concentration of bleach required to rapidly liquefy the sputa without partially dissolving the mites and consequently reducing their visibility under the microscope. Five dust mites were introduced into a Petri dish (diameter 10 cm) with 10 ml of commercial bleach (NaOCl 45 ml of active chlorine per litre). A second set of 5 mites was introduced into a Petri dish using the same commercial bleach, diluted by 50%, with water. Microscope observations were carried out at intervals of 10 min, 20 min, 1 h and 24 h. No difference was observed between the two types of bleach at 10 and 20 min. After 1 h a certain lightening and greater transparency was observed in those mites suspended in the concentrated bleach, although the dust mites were still visible. After 24 h, no dust mites were observed in the concentrated bleach; in the diluted bleach the mites were still visible, although poorly preserved. In view of these results, it was decided to use the diluted bleach for the liquefaction of the sputa in the subsequent part of this study.

Subsequently, 12 samples of sputa from different patients (3 serous, 3 mucous, 3 purulent and 3 hemoptoic), sent to the cytology laboratory for study, were contaminated with dust mites (5 in each sample). The dust mites were obtained by vacuuming mattresses

and carpets in bedrooms and living rooms. The mites were collected from slides of the vacuumed dust using a histological needle. The mites were then injected directly into each of the sputum samples.

The quantity of each of the sputum samples varied and, consequently, to provide a consistent ratio of bleach to sputum, 10 ml of diluted bleach was added for every 1 ml of sputum. For each type of sputa (serous, mucous, purulent and hemoptoc), one sample was left at room temperature for 24 h, one for 48 h and one for 72 h. Diluted bleach was added to liquefy the samples; and the time taken for complete liquefaction of the sample, assessed by visual inspection, was recorded. When the sputa had been liquefied, the resulting fluids were deposited in Petri dishes (diameter 10 cm) for observation under the microscope at low magnifications (40× and 100×).

Results

Table 1 summarises the types of expectorate examined, the duration of preservation of mites in sputa at room temperature, the time elapsed until total liquefaction of the sputum, the quantity of dust mites observed in each sputum sample, and the time employed for each microscope observation at low magnification (40× and 100×).

Liquefaction of the sputa with diluted bleach took longer for the purulent and hemoptoc sputa than for serous and mucous sputa. A greater quantity of froth was produced by the purulent or hemoptoc sputa, and in these samples it took longer for the bleach to act and for the froth to disappear. The fluid resulting from liquefaction of the serous or mucous sputa was transparent, whereas the sputa of purulent or hemoptoc appearance was more or less cloudy. The dust mites, however, were still clearly visible.

The proportion of dust mites identified varied according to the type of sputum. The best results were obtained with serous sputa (93.3%) compared to purulent sputa (60%). The results obtained for the sputa of mucous and hemoptoc appearances were 80 and 86.6%, respectively. Overall 48 of the 60 mites were detected, suggesting a test sensitivity of 80% (95% CI 68.2–88.2%).

Table 1 Types of sputa examined, duration of preservation of mites in sputa at room temperature, liquefaction time, quantity of dust mites observed and time employed in each microscopic observation

Type of sputa	Duration at room temperature (h)	Liquefaction time (min)	Number of dust mites observed	Duration of microscopic observation (min)
Serous	24	10	5 (100%)	2
	48	10	5 (100%)	2
	72	10	4 (80%)	2
Mucous	24	10	5 (100%)	2
	48	10	4 (80%)	2
	72	10	3 (60%)	3
Purulent	24	20	3 (60%)	3
	48	20	2 (40%)	4
	72	20	4 (80%)	4
Hemoptoc	24	30	5 (100%)	3
	48	30	4 (80%)	3
	72	30	4 (80%)	4
		Mean = 17.5	Total = 48 (80%)	Mean = 2.8

Less time was employed in observation under the microscope of the Petri dishes containing sputa of serous and mucous appearance (mean 2.1 min) than for those of purulent and hemoptoic appearance (mean 3.5 min).

Discussion

The sensitivity of our method for recovering dust mites was around 80%, although lower results occurred in the case of purulent sputa. This may have been because these samples had a more viscous consistency. It is also theoretically possible that not all the dust mites were injected into the sputa, but that some were retained in the needles used for inoculation.

Inhalation of particles is an inherent risk of respiration. Particles greater than 10 μm in diameter, including dust mites that have a size of around 350 μm , are usually deposited in the nasal passages or on the mucous blanket of the upper airways (Wang 1988). However, animal models indicate that mites can migrate into the lower airways, and pulmonary acariasis, characterised by respiratory symptoms and the presence of mites in the lungs, is well described in several species of monkey (Hirakoa et al. 2001). Human cases have been reported (Sasa 1950, 1951; Kijima 1963), particularly by Chinese researchers (Chen et al. 1990; Li and Li 1990; Xia et al. 2005). Clinical manifestations (productive cough, haemoptysis, chest pain, dyspnoea, etc.), and radiographic findings have been described, and Metronidazole has been effectively used in treating the condition.

A range of mite extraction methods have also been developed to identify dust mites (adult, larval or eggs) in other types of biological samples, such as in faeces and urine, for example, to identify cases of intestinal or urinary acariasis (Li et al. 2003; Dini and Freat 2005). Various compounds have been employed in these tests to extract dust mites in different substrata. Flotation methods have principally used concentrated lactic acid (Ciftci et al. 2006), a saturated solution of sodium chloride (Boquete et al. 2006), and dichloromethane (Racewicz 2001). However, to our knowledge, this study is the first to use bleach (NaOCl) to facilitate the detection of dust mites in samples of expectorate. The tests still requires familiarity with the cytological appearance of sputum samples, as mites may easily be mistaken for contaminants, particularly food particles.

The test we have piloted should facilitate the identification of mite species in sputum and may serve as the basis for further research into the relationship between mites, respiratory symptoms, and potential allergic responses in the respiratory tract. We believe that the method we have described for the liquefaction of sputum and the isolation and identification of dust mites will facilitate further research as the procedure can be performed quickly at room temperature, is simple, inexpensive, and less labourious than previous methods.

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