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Proteolytic enzyme sensitivity and decrease in respiratory function (a 10-year follow-up)

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Abstract Objectives: In workers, sensitivity and occupational asthma are known to develop in reaction to the proteolytic enzymes used in the manufacture of detergents. This study was conducted to find out how this sensitivity to the proteolytic enzymes affects respiratory function, excluding occupational asthma. **Methods:** Workers in the detergent industry ($n=65$) were divided into four groups according to their prick-test results, as follows: those positive for at least one enzyme (Lipolase, Savinase) (group I, $n=15$); enzyme-negative cases (group II, $n=50$); and from among these, enzyme-positive non-smoking cases (group Ia, $n=11$); enzyme-negative non-smoking cases (group IIa, $n=32$). Respiratory function tests for the last 10 years from the archives were assessed. For statistic analysis, average values were determined and the standard deviation calculated. For comparison of the groups, the Mann-Whitney U and Fischer Exact chi-square tests were used. **Results:** The ratio of smokers, the cigarette burden, average age and the period of work were found to be similar between workers of groups I and II, and group Ia and group IIa ($P>0.05$). The average annual fall in the forced vital capacity (FVC) and the forced expiratory volume in the first second (FEV₁) for the 10-year period was, respectively, found to be: group I, 64.1 ± 7.8 ml and 58.7 ± 9.6 ml; group II, 38.7 ± 6.4 ml and 43.7 ± 8.2 ml; group Ia, 60.7 ± 8.1 ml and 56.1 ± 10.2 ml; group IIa, 37.4 ± 6.8 ml and 42.9 ± 8.1 ml. The decrease in FVC and FEV₁ in group I compared with group II, and in

group Ia compared with group IIa was statistically significant ($P<0.001$). **Conclusions:** In this study, the observation that the FVC and FEV₁ of the workers were much lower than expected, especially among those sensitive to the enzymes, independently of occupational asthma and cigarette smoking, led to the conclusion that it could be due to sensitivity to the enzymes.

Keywords Proteolytic enzyme · Respiratory function test · Prick test

Introduction

Proteolytic enzymes from *Bacillus subtilis* were introduced into detergent products in the 1960s. Within a few years of their introduction, several investigators reported the association of exposure to detergent enzymes with asthma, and nasal and ocular symptoms among workers involved in the manufacture of detergents [1, 2].

In later years, however, more studies investigating sensitivity to the proteolytic enzymes, the ratios of the occupational asthma cases due to these enzymes, and the relationships between industrial technology and sensitivity and occupational asthma were reported [3, 4]. The majority of these studies were cross-sectional analyses that examined either the sensitivities to these proteolytic enzymes or together with the ratios of occupational asthma.

In prospective studies based on follow-up of the respiratory function tests, no significant fall in the respiratory function tests of the detergent workers, or that the fall might be related to the differences in the smoking habits of the workers, was suggested [5]. In contrast to this, Pepys et al. [2] conducted provocative inhalation tests with the enzymes and observed a fall in the carbon monoxide diffusion test in some workers, which led them to conclude that this was due to the fact that the proteolytic enzymes affected the airways down to the level of the pulmonary parenchyma.

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Today, though proteolytic enzymes are known to induce allergic rhinitis, occupational asthma, and extrinsic allergic alveolitis, the long-term effects of the enzyme sensitivity alone has not been studied well. The aim of this study was to investigate the long-term effect of the sensitivity on respiratory function, excluding occupational asthma or extrinsic allergic alveolitis, due to proteolytic enzymes among workers in the detergent industry.

Materials and methods

This study was conducted on workers in a detergent factory, between May and June of 1995. The respiratory complaints of each worker were recorded, physical examinations conducted, and P-A chest radiographs taken and interpreted by a radiology specialist. Past medical history, physical examination, peak expiratory flow rate (PEFR) records, and respiratory function tests were conducted, and those workers diagnosed with occupational asthma were excluded from the study. The respiratory function test was done by following the instructions of the ATS vitalography equipment and using the dry spirometer (Vitalograf, England) to measure the forced vital capacity (FVC) and forced expiratory volume in the first second (FEV₁) [6]. Since 1986, under the supervision of the work-site physician, a health officer has recorded the respiratory function tests of the workers in May and June of every year and kept them in the company health records.

The prick test was performed on the forearm with nine allergens obtained from Allergopharma Joachim Ganzer, Hamburg, Germany (Graser/getralide, Graser, Baumel, *Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*, Milbe 1, Milbe 2) and material containing Lipolase, Savinase. As a positive control histamine phosphate was used, while physiological serum was used as the negative control. In the prick test, erythema above 3 mm was considered to be positive [7]. In the prick test, two groups, those sensitive to the enzymes used in the factory (group I) and those not sensitive (group II) were formed. To eradicate the effect of cigarette smoking on respiratory function the non-smokers were further divided into an enzyme-positive group (group Ia) and an enzyme-negative group (group IIa).

In the statistical analysis, the average value and standard errors were calculated for each worker. Comparison of the groups was by the Mann-Whitney U and Fischer Exact chi-square tests, with $P < 0.05$ considered to be significant.

Results

Ninety-seven workers were employed in the detergent factory. Fourteen workers were not included in the study because of pre-existing occupational asthma ($n=9$), chronic obstructive lung disease ($n=3$), bronchiectasis ($n=1$) and advanced kyphoscoliosis ($n=1$). Later on, 18 patients were excluded from the study during the 10-year follow-up, because of incomplete data ($n=4$), leaving the company for non-medical reasons ($n=11$) and death ($n=3$). Of the 65 workers who completed the study, 61 were male and four were female. The average age of the workers was 37.6 ± 5.8 years, and the average period of working at the factory was 13.7 ± 4.2 years. Sixteen workers had prick-test positivity for more than one of the usual aeroallergens. The number of cigarette smokers was 22, with average years of smoking and estimated cigarette burdens of 13.7 ± 4.3 years and 14.5 ± 5.6 pack-years, respectively. The characteristics of the workers in the study are shown in Table 1. The ratio of smokers in

Table 1. Characteristics of the workers in the groups

Characteristic	Group I	Group II	Group Ia	Group IIa
Total number of cases	15	50	11	32
Male	12	41	10	28
Female	1	3	1	2
Number of smokers	4	18	—	—
Average age (years)	37.1 ± 5.9	37.7 ± 5.8	36.3 ± 6.0	38.0 ± 5.7
Working time (years)	12.4 ± 4.1	14.1 ± 4.2	12.5 ± 4.6	14.6 ± 4.2
Prick test positivity ^a	6	10	4	7

^aPositive to at least one of the aeroallergens

groups I and II and their cigarette burdens, average ages and the number of years worked was found to be similar ($P > 0.05$).

In the enzyme-positive cases, sensitivity to other aeroallergens was found to be significantly higher than those of the enzyme-negative cases ($P < 0.001$). The chest radiographs of the workers were interpreted as being normal.

The annual fall in FVC and FEV₁ in the respiratory function tests was: group I, 64.1 ± 7.8 ml and 58.7 ± 9.6 ml; group II, 38.7 ± 6.4 ml and 43.7 ± 8.2 ml; group Ia, 60.7 ± 8.1 ml and 56.1 ± 10.2 ml; group IIa, 37.4 ± 6.8 ml and 42.9 ± 8.1 ml. The decrease in FVC and FEV₁ was significantly greater in group I than in group II and in group Ia more than in group IIa ($P < 0.001$). The fall for all the groups, however, was, respectively, found to be 44.6 ml and 47.1 ml annually. The rate of fall in the FVC and FEV₁ for the 10-year period is shown in Fig. 1.

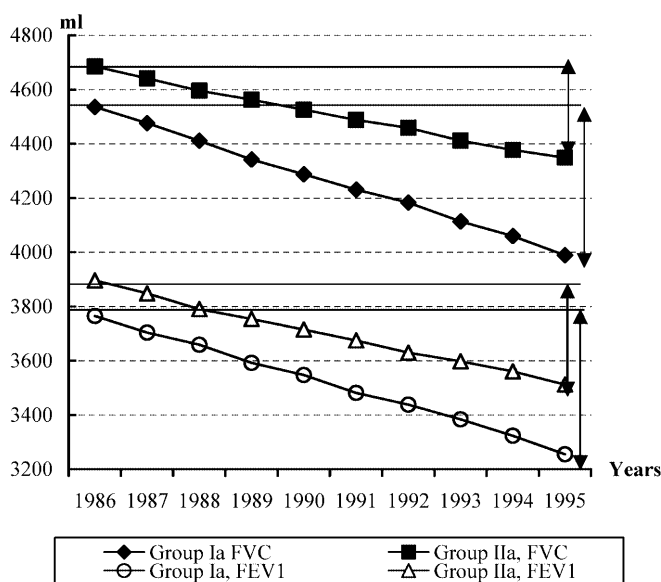


Fig. 1. Decline rate of FVC and FEV₁ for the enzyme-positive (group Ia) and enzyme-negative (group IIa) cases among non-smoking workers

Discussion

In this study, sensitivity to proteolytic enzymes was found to increase the annual fall in FVC and FEV₁ independently of occupational asthma or smoking habit.

Proteolytic enzymes used in the production of detergents are known to cause occupational asthma and pulmonary parenchymal diseases. For this reason, measures were taken to reduce the dust and enzyme concentration at the work environment, and the proteolytic enzymes were passed through a chemical process and encapsulated. In a study, despite the use of encapsulated enzyme preparations, it was stated that the possibility of high concentrations of the enzymes in the environment could have led to the rather high sensitivities observed in contrast to what was expected [8]. Work by Cullinan et al. [9] supports the fact that to prevent allergy and asthma due to the enzyme, encapsulating the enzyme alone was not enough. Thus, in our study, despite enzyme encapsulation, 23% of the cases were still sensitive to the enzyme.

Reports from Australia show that in those workers who had been severely exposed to the enzyme in the past, there was a decrease in the elastic recoil of their chest [10]. However, a case of extrinsic allergic alveolitis with a restrictive respiratory pattern resulting from proteolytic enzymes was reported [11]. Our cases had neither emphysema-like finding nor an alveolar interstitial pattern on the chest radiograph.

Nicholson et al. [12] recommend health checks for employees of detergent and soap factories once every 6 months for the first 2 years and, subsequently, once a year. Here, the aim is the early diagnosis of these occupational diseases. However, the known diseases in the detergent industry include allergic rhinitis, occupational asthma, and rarely, the alveolar or interstitial patterns seen on chest radiographs. In this study however, it was noted that without these known diseases, there was still a more-than-expected fall in respiratory function. In another study on 121 workers exposed to *Bacillus subtilis*, assessment of enzyme sensitivity and pulmonary function tests found FEV₁/FVC in 21 of the 48 sensitive patients, and ten of the 73 non-sensitive workers, to be under 70%. The investigators repeated the same tests 6 months later and found that negative enzyme sensitivity could turn positive, despite the breaking of its relationship with *Bacillus subtilis* enzymes. The derangement in the pulmonary function tests could be severe and irreversible [13].

Studies to examine the respiratory function of the workers exposed to proteolytic enzymes were also carried out. Cathcart et al. [14] followed FEV₁ and FVC for 4–20 years at five different centers in England and found that significant falls were related to the geographic location and smoking habit. They failed to establish any relationship between the proteolytic enzymes and respiratory function. In our study, after the exclusion of the negative effects of cigarette smoking on respiratory function, and to make sure that only the effect of enzyme

positivity on respiratory function was observed, we divided groups I and II into groups Ia and IIa by excluding smokers from these groups. In terms of the fall in FVC and FEV₁, there was a statistically significant difference between the two groups ($P < 0.001$). Flood et al. [5] in their study on workers from a detergent factory, suggest that enzyme exposure has no effect on pulmonary function. In an 11-year study, an average annual fall of 39 ml in FEV₁ was found. In a study in which periodic pulmonary function tests were carried out on 2,000 workers, the rate of fall of FEV₁ was found to be 45 ml per year. This value was similar in workers in other industries not related to detergent production [15]. In our study, the observed average annual fall in FVC was 64.1 ± 7.8 ml in group I, while that for FEV₁ was 58.7 ± 9.6 ml. In enzyme-sensitive non-smoking workers however, the average fall in FVC per year was 60.7 ± 8.1 ml and that of FEV₁ was 56.1 ± 10.2 ml. These values are higher than the 10–35 ml annual decline in the non-smoking healthy general population. On the other hand, as shown in Fig. 1, the enzyme-positive non-smoking group had a faster annual decline, approximately 20 ml in FEV₁ and 25 ml in FVC, than the enzyme-negative non-smoking group. In other words, enzyme sensitivity doubled the natural decline, independently of smoking. From this, it is not difficult to say that some of these enzyme-positive employees will demonstrate respiratory symptoms in later life.

Gottlieb et al. [16], in a study to test the hypothesis that allergy leads to chronic irreversible airway obstruction, and carrying out respiratory function tests on average every 3.1 years, found that the group sensitive to the allergens had statistically significant decreases in their respiratory functions. Postma et al. [17], in a study conducted on patients with chronic obstructive pulmonary disease (COPD) who were not on steroids, found an annual fall in FEV₁ of 82 ml/year. The enzyme sensitivity among workers in the detergent industry can be said to cause a decrease in respiratory function similar to that of COPD.

In conclusion, in the 10-year period, the more-than-expected fall in FVC and FEV₁ in respiratory function tests of the workers, especially in the enzyme-positive group, without apparent respiratory complaints, led to our conclusion that this fall increased with enzyme sensitivity, independently of occupational asthma.

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