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Chronic Lung inflammation in victims of toxic gas leak at Bhopal

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Bronchoalveolar lavage (BAL) studies in 20 patients at Bhopal. 1.3 ± 0.4 yr and 2.7 ± 0.6 yr after toxic gas exposure had revealed that the lower respiratory tract inflammation had progressed from initial macrophage alveolitis to macrophage-neutrophilic alveolitis. The interval between the two lavages was 1.4 ± 0.6 yr. BAL studies in a new group of 14 patients 5.1 ± 1.0 yr after exposure had confirmed chronic inflammation of the lower respiratory tract as evidenced by macrophage-neutrophilic alveolitis in these subjects as well. Clinical. radiographic and pulmonary function abnormalities were persistent in a proportion of subjects in both groups.

Fibronectin (FN) levels were estimated in BAL fluid in 41 patients. Elevated FN levels were seen in 12 (29.3%) subjects and nine of these 12 had radiographic abnormalities. Severely exposed subjects (n=30) had significantly higher BAL fibronectin levels compared to normal subjects and mild/moderately exposed subjects. Repeat FN estimations in BAL samples from 10 patients had revealed that five had abnormally high FN including three who had high FN on both occasions. The number of patients showing abnormal decline in pulmonary function was higher in patients with elevated FN than in patients with normal FN.

Thus, persisting clinical, roentgenographic and ventilatory abnormalities, as well as macrophage-neutrophilic alveolitis along with abnormally elevated FN levels in a proportion of subjects, suggest the possibility that lung fibrosis can occur in subjects exposed to toxic gas at Bhopal.

Introduction

Bronchoalveolar lavage (BAL) studies in toxic gas exposed subjects 1.5 ± 0.6 yr after exposure (1985-1987) had revealed that there was a subclinical alveolitis characterized by accumulation of macrophages in the lower respiratory tract, especially in severely exposed subjects (1). It was not certain from this study whether the inflammatory cells recovered from the lower respiratory tract were activated; and if so, is there any evidence of release of markers of activity such as fibronectin (FN) by these cells into the lavage fluid? It is possible that the macrophage alveolitis may have spontaneous remission, as it had been shown in pneumoconiosis that initial macrophage alveolitis did not necessarily lead to interstitial fibrosis (2). However, there is also a possibility that the cells comprising alveolitis may release toxic mediators (3), resulting in injury and fibrosis to lung parenchyma. A study was. therefore, planned to investigate the fate of alveolitis in patients from the first study (1) by relavaging them 2.7 ± 0.6 yr (1987-1989) after exposure and also to evaluate changes in the lower respiratory tract in a new group of patients 5.1 ± 1.0 yr (1988-1991) after exposure. The study was further utilized to investigate the presence of markers of activity from inflammatory cells by estimating FN concentrations in BAL fluid.

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Subjects and Methods

This study involving 44 patients was done 2-7 yr (1987-1991) after exposure to the toxic gas. All studies were carried out under protocols approved by the Indian Council of Medical Research, New Delhi. Informed consent was obtained from each subject after explaining the procedure in local language.

SUBJECTS

Twenty out of 36 patients studied initially during 1985-1987 (1) were willing for relavage studies. The first lavage in these 20 patients was carried out

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1.3 ± 0.4 yr after exposure. Relavages were carried out during 1987-1989 ie. 2.7 ± 0.6 yr after exposure and they are designated as Group 1 subjects. The interval between the two lavages was 1.4 ± 0.6 yr. A new group of 24 patients was also studied during 1988-1991 i.e. 5.1 ± 1.0yr after exposure and is classified as Group 2. The selection criteria for Group 2 patients were similar to those for the initial study (1). Briefly, all patients were residents of an area near the Union Carbide factory (within 5 km) and were exposed to the toxic gas on the night of December 2-3, 1984. All had respiratory and ophthalmic symptoms on the day of exposure and continued to have respiratory symptoms at the time of evaluation. Main symptoms were cough, exertional dyspnoea and chest pain. None of them had any previous cardio-respiratory disease as assessed intensively by history. physical examination and skiagram chest. Patients with the slightest doubt of pre-existing lung disease were excluded from the study.

CONTROL SUBJECTS

Seventeen non-smoking individuals (age 28.8 ± 8.5 yr) were studied as control subjects for comparison with BAL cellular profile. They were unexposed to the toxic gas and were residents of Madras city. For comparison of BAL fluid fibronectin, 10 non-smoking, normal subjects, not exposed to the gas and residing at Delhi were evaluated. None of the control subjects had respiratory symptoms or abnormal physical findings and all had normal chest radiographs. Pulmonary function measurements in control subjects were similar to those of predicted values (4). None of the normal subjects was on any medication.

PRE-LAVAGE ASSESSMENT AND INVESTIGATIONS

Pre-lavage assessment and investigations of each patient included detailed history, physical examination, a full-plate PA chest roentgenogram a 12 lead electrocardiogram, total and differential leucocyte count in the peripheral blood and pulmonary function tests such as FVC, FEV_1 and FEV_1/FVC% (using Transfer Test Model C, PK Morgan Pvt Ltd, Chatham, U.K.). Patients with pre-existing lung diseases were excluded from the study.

BRONCHOALVEOLAR LAVAGE

Bronchoscopy and bronchoalveolar lavages (BAL) were carried out as an outpatient procedure as Hamidia Hospital, Bhopal, as previously described (1). In brief, the flexible fibre optic bronchoscope was passed either transnasally or transorally and the lavages were done from three subsegments viz. right middle lobe, lingula and left lower lobe. BAL was performed with 300ml sterile 0.9% saline at room temperature. One hundred millilitres of sterile saline in five 20 ml aliquots was infused through the fibre optic bronchoscope into each of the three lobes in the lower respiratory tract. After each aliquot was infused, the cells and lavage fluids were recovered by gentle suction using 50-100 mm H_2O negative pressure with a clinical suction apparatus and collected in the specimen traps (Sherwood Medical. Ireland). The cells recovered by lavage were counted on a haemocytometer, using the unconcentrated lavage fluid (5) and expressed as cells dl^{-1} of recovered fluid. The lavage cell differentials were determined using filtration method (5). Using oil immersion of a microscope, alveolar macrophages, lymphocytes, neutrophils and eosinophils were identified and 400 cells were counted from each preparation for deriving the differentials by two independent observers and recorded independently. Both observers agreed to within 5% of all lavages and the mean value was used for analysis. Epithelial cells counted were always less than 5%. Absolute numbers of different types of cells were derived from total cells times differential percentages.

FIBRONECTIN ESTIMATION

Fibronectin (FN) and albumin in BAL fluid were estimated in 41 patients including 31 patients from the initial study (1). Thirty patients had severe exposure and 11 had mild/moderate exposure and the severity of exposure was determined as per previously defined criteria (1). Ten severely exposed patients and one mild/moderately exposed patient were smokers. Ten patients from the initial study had two FN estimations from two lavages on different occasions. The interval between the two lavages was 1.6 = 0.7 yr. Fibronectin was measured by simple radialimmunodiffusion (6) with immunodiffusion plates (LC Pamigen, Behering Diagnostics, Germany). Albumin estimation was done by spectrophotometric method (6). FN in BAL fluid was expressed as $\mu g\ mg^{-1}$ albumin.

ANALYSIS

Statistical analysis was done using Mann-Whitney U-test for independent groups and Wilcoxon Signed Rank-test for paired observations.

Fibronectin values more than two standard deviations above the mean of normal subjects (i.e. $\geq 10\ \mu g\ mg^{-1}$ albumin) were classified as abnormally high. Odds ratio was calculated to find the relative risk of severity of exposure with elevated FN levels. If either FVC or FEV_1 decreased by more than

10% during the second lavage, patients were characterized as 'deteriorated'. If none of the parameters decreased by more than 10% or any parameter increased by more than 10%, they were classified as 'no change or improvement' (7).

Results

GROUP 1 PATIENTS

All patients in this group (n=20) were males. The mean age was 38.5 ± 9 yr. Three patients continued to smoke though with reduced intensity, despite repeated medical advice to quit smoking. Respiratory symptoms such as cough, exceptional dyspnoea and chest pain were persisting in all patients at the time of the second lavage. Four patients had rales/rhonchi at initial study. Signs persisted in three and disappeared in one. However, three developed fresh signs, making a total of six patients with rales/rhonchi at the time of the second lavage. Radiographic abnormalities of 1/0 to 2/1 (ILO, 1980 classification) (8) were observed in nine out of 20 patients. Although there was no significant difference in pulmonary function measurements (FVC and FEV_1) between the first and second lavages, these continued to be significantly lower than the normal predicted values (9,10) during the second lavage (Table 1).

BAL results during the first and second lavages in Group 1 patients are shown in Table 2. Total inflammatory cells (P=0.004) and absolute (total) number of alveolar macrophages (P=0.003) were significantly higher in patients compared to normal subjects during the first lavage, despite significantly lower fluid recovery (normal subjects *vs.* Group 1 patients; 60.1 ± 7.0 ml, 44.6 ± 13.2 ml. P=0.008). During the second lavage, the total inflammatory cells (P=0.004) and total macrophages (P=0.002) continued to be significantly higher than normal subjects. In addition. there was a significant rise in the neutrophil proportion (P=0.03) and total neutrophils (P=0.03). In comparison with results of the first lavage, a significant rise in neutrophil percentage (P=0.02) and total neutrophils (P=0.04) was also seen during the second lavage. There was no significant difference in the fluid recovery between the first and the second lavage (44.6 ± 13.2 *vs.* 48.3 ± 12.4, P>O.2).

GROUP 2 PATIENTS

Of the 24 patients in this group, six were females and two males were smokers. The mean age was 34.1 ± 9.5 yr. All patients had persistent respiratory symptoms such as cough, dyspnoea on exertion and chest pain and three had rhonchi. Radiographic abnormalities of 1/0 to 2/2 were observed in 15 out of 24 patients. FVC and FEV_1 were significantly lower in this group as well as compared to the normal predicted values (9,10) (Table 1).

Table 1 Pulmonary function results

	Observed	Predicted	% Predicted
Group I (n=20)			
(a) First BAL			
FVC:	3.08* ± 0.82	3.83 ± 0.43	80.4 ± 19.7
FEV_1:	2.45* ± 0.77	3.06 ± 0.34	80.0 ± 23.0
FEV_1/FVC%	79.2 ± 11.5	–	–
(b) Second BAL			
FVC:	3.19* ± 0.70	3.80 ± 0.45	83.3 ± 14.0
FEV_1:	2.40* ± 0.73	3.06 ± 0.34	78.4 ± 20.4
FEV_1/FVC%	74.7 ± 13.2	–	–
Group 2 (*n* = 24)			
FVC:	3.12** ± 0.92	3.53 ± 0.67	87.5 ± 18.0
FEV_1	2.46*** ±0.84	2.82 ±0.53	87.1 ± 25.1
FEV_1/FVC%	80.5 ± 13.3	–	–
Control subjects (n= 17)			
FVC:	3.08 ± 0.59	3.16 ± 0.52	97.3 ± 12.9
FEV_1:	2.70 ± 0.59	2.81 ± 0.54	95.2 ± 14.8
FEV_1/FVC%	87.1 ± 7.3	–	–

Group 1: Patients with first and second BAL.
Group 1: New series of patients with BAL.
P value, * <0.001, ** <0.01, *** <0.05.

The mean values of total inflammatory cells (P=0.04), total alveolar macrophages (P=0.03), neutrophils percentage (P=0.009) and total neutrophils (P=0.007) were significantly higher in this group of patients compared to normal subjects (Table 2). The mean fluid recovery was similar in normal subjects and patients (normal 60.1 ± 7 ml *vs.* 56.0 ± 8.8 ml, P=0.1).

FIBRONECTIN LEVELS

The mean FN level in 10 non-smoking normal subjects was 6.31 ± 1.83 μg mg^{-1} albumin. The mean FN level in severely exposed patients (n=30), 13.2 ± 11.1 μg mg^{-1} albumin, was significantly higher (P=0.04) compared to normal subjects and this was true when only non-smokers (n=20) were

Table 2 Bronchoalveolar lavage results

	Normal subjects (n=17)	Group 1		Group 2 (n=24)
		First lavage (n=20)	Second lavage (n=20)	
Total cells	15.8	28.0*	31.6*	26.4†
$\times 10^6$ dl^{-1}	± 6.7	± 15.2	± 20.3	± 20.2
Macrophages				
%	83.9	88.3	87.7	89.4
	± 6.4	± 8.0	± 7.0	± 11.0
$\times 10^6$ dl^{-1}	13.2	25.3*	27.6*	24.1†
	± 5.6	± 15.3	± 17.8	± 19.9
Lymphocytes				
%	14.4	10.2	8.0*	7.1†
	±6.4	± 7.6	± 5.1	± 10.0
$\times 10^6$ dl^{-1}	2.4	2.4	2.6	1.5
	± 1.5	± 2.0	± 3.3	± 2.5
Neutrophils				
%	0.7	1.1	3.6**	2.1‡
	± 0.8	± 1.4	± 4.5	± 1.8
$\times 10^6$ dl^{-1}	0.1	0.3	1.2**	0.5‡
	± 0.1	± 0.3	± 2.1	± 0.6
Eosinophils				
%	1.0	0.4	0.8	1.4
	± 1.1	± 1.8	± 1.6	± 2.6
$\times 10^6$ dl^{-1}	0.1	0.1	0.2	0.3
	± 0.1	± 0.4	± 0.4	± 0.5
% Recovery	60.1	44.6*	48.3*	56.0
	± 7.0	± 13.2	± 12.4	± 8.8

P values
Group 1
*<0·01 as compared to normal subjects.
**<0·05 as compared to normal subjects and 1st lavage.
Group 2
†<0·05, ‡<0·01 as compared to normal subjects.

considered (P=0·03). In mild/moderately exposed patients (n=11), the mean FN level, 7·0 ± 2·9 μg mg^{-1} albumin was similar to normal subjects (Table 3). Twelve out of 41 patients (29·3%) had elevated FN levels in BAL fluid. Of these 12 patients, 11 had severe exposure and one had moderate exposure. Severely exposed subjects had six times (odds ratio=5·8) higher risk of having increased FN levels than mild/moderately exposed subjects. Radiographic abnormalities of 1/0 or greater were observed in 23 out of 41 patients. The mean FN level (12·9 ± 11·0 μg mg^{-1} albumin) in the radiologically abnormal group (n=23) had shown a trend towards higher values compared to the mean FN values (9·9 ± 8·0 μg mg^{-1} albumin) in the radiologically normal group (P=0·08). Radiological abnormalities were seen in nine of 12 patients with elevated FN levels and 14 of 29 with normal FN levels.

In 10 patients who had FN estimations in two lavage samples at different time points, there was no significant difference on FN levels (first lavage 13.0 ± 9.6 μg mg^{-1} albumin *vs.* second lavage 13.3 ± 11.8 μg mg^{-1} albumin, P>0.2) (Table 3). Of these 10, four had elevated levels during the first lavage and three continued to have elevated levels during the second lavage. One patient who had normal FN during the first lavage had an elevated level during the second lavage. Another subject with elevated levels during the first lavage had shown a reduction to normal values during the second lavage. Thus, five subjects had elevated FN levels at any one occasion. Of the five patients who had elevated FN levels, three had functional deterioration and two had no change. All were non-smokers. On the other hand. of the five subjects who had normal FN levels on both occasions, two deteriorated and one was a smoker; one unproved and two had no change. Thus, three of five subjects who had elevated FN at any one occasion had a decline in pulmonary function, whereas only one of four subjects (excluding smoker)

Table 3 Fibronectin levels in gas exposed subjects

	Control subjects (n= 10)	Exposure		Radiology		Pulmonary function changes between two lavages (n= 10)		
		Mild/moderate (n = 11)	Severe (n = 30)	Normal (n= 18)	Abnormal (n=23)	No change (n=4)	Improv ement (n=1)	Deterioration (n=5)
(a) Mean FN level ($\mu g\ mg^{-1}$ albumin)	6.31 ± 1.83	7.0 ± 2.9	13.2* ± 11.1	9.8 ± 8.0	12.9** ± 11.0	–		
(b) Patients with								
Normal FN	10	10	19	15	14	2		2†
Elevated FN	0	1	11†	3	9	2		3

FN: fibronectin, †, one patient was a smoker.
P values, *, 0·04; **, 0·08, compared to control subjects.
‡, Odds ratio between mild/moderate and severely exposed 5·8.

with normal FN on both occasions had a decline in pulmonary function.

Discussion

The observation that the lower respiratory tract inflammation has progressed from macrophage alveolitis to macrophage-neutrophiic alveolitis 2.7 ± 0.6 yr after toxic gas exposure and the finding of macrophage-neutrophilic alveolitis in a new cohort of patients during 1988-1991 (5.1 ± 1.0 yr after exposure) suggests that chronic inflammation of the lower respiratory tract is a feature of toxic gas-induced lung disease. The fact that alveolar macrophages and neutrophils are capable of promoting lung injury and fibrosis (11) suggests the possibility that inhaled toxic gas may produce permanent damage to pulmonary parenchyma. Abnormal accumulation of macrophages with or without neutrophils has been described in various chronic lung diseases (12-14) and also in experimental studies in animals exposed to asbestos fibres (15,16). It has also been shown in idiopathic pulmonary fibrosis that activated alveolar macrophages can produce neutrophil chemoattractants (17.18). Therefore, we believe that the progression of initial macrophage alveolitis to macrophage-neutrophic alveolitis represents the stages in the evolution of 'toxic gas induced lung disease'. Thus, the persistence of clinical, radiological and pulmonary function abnormalities in this study as well as in previous studies (19-21) may be due to the consequences of macrophage-neutrophilic alveolitis.

Abnormally elevated FN levels in BAL fluid were observed in nearly 30% of patients in this study and a higher proportion of severely exposed subjects had elevated FN levels compared to mildly/moderately exposed. Elevated levels of FN in BAL fluid had been described in interstitial lung diseases such as sarcoidosis and idiopathic pulmonary fibrosis (22-24) and it had been shown that activated alveolar macrophages were capable of producing FN (25). Therefore. the observation of elevated levels of FN in a good proportion of subjects in this study may suggest that the expanded numbers of inflammatory cells especially alveolar macrophages are activated. Fibronectin along with other mediators had been shown to contribute to the development of fibrosis by acting as a chemoattractant to fibroblasts in interstitial lung diseases (11,25). Therefore, the findings of elevated levels of FN in BAL fluid with macrophage-neutrophilic alveolitis may suggest that some of these patients may develop lung fibrosis. This has been further corroborated by the observation that three of five non-smoking subjects who had elevated FN levels at any one point of time had a decline in pulmonary function. In conformity with this, pulmonary fibrosis had been demonstrated by computerized tomography (CT) in a toxic gas exposed victim (26) and in experimental animals exposed to methyl isocyanate (27).

As some of the patients exposed to toxic gas at Bhopal are having persistent respiratory symptoms, roentgenographic abnormalities and ventilatory defects as evidenced by significantly reduced pulmonary function values even 5 yr after exposure, the persisting alveolitis may result in further functional lung derangements that may lead to respiratory crippling. Therefore preventive and rehabilitative modalities of treatment for suppression of alveolitis are needed in this population.

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