Silicosis is a well known industrial occupational lung disease of the workers dealing with quartz dust. Development of silicosis depends on inhalation of respirable free silica particles <10 μm in diameter. Acute silicosis is a rare condition related to exposure of single heavy concentration of respirable free silica leading to inflammation and consolidation of lung. Chronic silicosis is due to reactions of respirable silica with pulmonary tissue chronically exposed for 20 years or more with concentration of quartz in the air less than 30 %. There are pulmonary inflammatory changes with formation of nodular fibrotic lesions. Initial injury is usually followed by an acute exudative response, in which proteins and early inflammatory cells, mainly neutrophils, move into the injured area. Persistence of the injury results in a shift to more chronic inflammatory cells (mononuclear cells) and may lead to fibrosis. With the combined use of serial bronchoscopy, biopsy, and bronchoalveolar lavage (BAL), important distinctions among the various inflammatory process in pulmonary parenchyma are emerging. The type of cells obtained by total pulmonary lavage from experimental animals is similar to those recovered by segmental lavage in humans. The type and number of cells found in lavage fluid are similar to those found in biopsy specimens of the lungs. The development of nodular and peribronchial fibrosis following inhalation and intratracheal injection of silica and resemblance of these patterns with that of human silicosis have been well documented. Still the pathogenesis of silicosis is under critical review. The present study has been undertaken to find out early biological marker for diagnosis of silicosis and to document the time-course relationship between the introduction of quartz into the respiratory system and the subsequent development of inflammatory cellular changes in BALF and histopathological changes in the lungs in two models of silicosis in rats.

Materials and Methods

In order to document duration dependent changes two experimental models of rats were included in the study — (i) acute model for documentation of early changes; (ii) chronic model for documentation of late or chronic changes. These acute and chronic models were produced by intratracheal injection and inhalation of quartz (silica) respectively. Quartz in the form of granules was supplied by LOBA CHEMIE, India. This marketed product contains silicon dioxide (quartz) granules of 0.2-0.8mm in size. Quartz dust was then obtained from those granular quartz ground into fine powder in the laboratory.

Inbred wistar rats of either sex (150-200 g) were maintained under standard animal house condition.
and supplied with food and water ad libitum. The rats were observed for one week prior to experimentation for any sign of illness. Sixty rats for intratracheal injection (acute) model and another 60 rats for inhalation (chronic) model were selected. The rats of each model were taken as control vs treated (quartz exposed) of 30 each. Both the control and treated rats of each model were divided into 3 groups of 10 rats each according to the durations of exposures (inhalation) and post exposure periods (intratracheal injection).

Intratracheal injection model—The rats were anaesthetised with sodium pentobarbitone (50mg/kg, ip). Trachea was exposed by making a midline incision in neck. Intratracheal injection (through 24 size needle) of 10mg quartz suspended in 0.05ml saline was given to the animals of the treatment group. The control groups received only 0.05 ml saline following the same procedure. After closing the wound the rats were kept for 3, 5 or 7 days after the single intratracheal administration of quartz or saline respectively for treated and control groups and accordingly animals were divided into those 3 groups.

Inhalation model—In the treatment groups of the inhalation model the rats received quartz dust with air flow 5l/hr in a simulation chamber (concentration of quartz 40mg/m³, 50±10% RH and 22±2°C) for 6 hr a day, 6 days a week. The quartz dust was stored in a conical flask where the dust was diluted with flow of clean air of 5 l/hr through a motor. The resulting aerosol was delivered to the exposure chamber with the jet air flow. The air (aerosol) supplied to the inhalation chamber was filtered through high efficiency particulate air filters by an air sampler to measure the concentration of the dust in the chamber by estimating suspended particulate matter (SPM).

$$\text{SPM} = \frac{W_f - W_o}{T \times R} \times 1000$$ in m³ area

where $W_f$ = final weight of the filter paper in mg; $W_0$ = initial weight of the filter paper in mg; $T$ = time of sampling in min; and $R$ = rate of air flow in l/min.

Thus the concentration of quartz dust in the inhalation chamber was obtained as 40 mg/m³. The control groups received only the fresh air in the chamber under the simulatory conditions. The inhalation was given for a period of 2, 4 or 8 weeks and accordingly animals were divided into those 3 groups.

Collection of BALF—Following the final exposure (for inhalation model) and on completion of post exposure periods (for intratracheal injection model), the rats of both treated and control groups were anaesthetised with urethane (1.5gm/kg, ip). Exposing trachea with midline incision in neck a 12-gauge plastic cannula was introduced into the trachea and lavaged with phosphate buffer saline with 2ml aliquot for five times. Thus a total of 10 ml fluid was introduced with the help of a syringe. The lavagates were aspirated gently each time until slight negative pressure was felt on the syringe plunger. From each rat average 6-7ml of BALF was recovered and kept for cytological analysis.

Cytology—Total and differential counts of inflammatory cells in BALF were done to observe the changes in their number and morphology. Total cells were counted directly with BALF mixed (ratio 1 : 2) with dilution fluid of 2% acetic acid and 2.6% methylene blue in a haemocytometer (Neubaur's) chamber³. The results were expressed as the number of cells per ml of BALF.

After the total cell count the BALF was centrifuged at 1500 rpm for 10 minutes to get the cell pellet. The supernatant fluid was removed. Cell pellet was smeared and stained with Jenner-Giemsa method on the slide. The differential cell count was done under oil immersion lens. The differential count was expressed as percentage of cellularity.

Histopathology—After the collection of BALF, the rats were sacrificed and lungs were dissected out. The macroscopic findings were noted and the lungs were fixed by immersing and preserving them in 10% formalin. The left and right lobes were cut in sagittal plane and sections were examined for macroscopic evidence of consolidation, fibrosis or nodulation. Sections from both the lungs were subjected to routine paraffin processing. Sections (4-5 μm thick) were cut and the slides were stained with hematoxylin and eosin for microscopical examination. Conditions of interstitium, alveoli, bronchi and blood vessels were noted on light microscopy.

Statistics—The results of the total and differential cell counts were evaluated statistically by Student's $t$ test. At $P<0.05$, results were considered as statistically significant. All the data were presented as mean and standard error (mean±SE). For histopathology no statistical analysis was made.

Results

Cytology—There was a highly significant ($P<0.001$) increase in the total number of the BALF cells in all the experimental groups as compared to the
control animals (Table 1). The increase in cell counts was four to five fold in quartz exposed intratracheal injection and inhalation groups as compared to controls. Differential cell count also showed a highly significant ($P<0.001$) change between the control and the treated animals (Tables 2 and 3).

In the treated groups the alveolar macrophages on differential count were of two types: foamy macrophages with vacuolated cytoplasm and large sized tingible body macrophages containing engulfed particulate matter. The cell number per high power field (oil immersion field; x 1000) was 2-5 and 25-30 cells per field in control and treated rats respectively. There were more densely populated cells in the inhalation groups than the intratracheal groups. In the treated groups occasional bronchial epithelial cells, giant and plasma cells, and eosinophils were also seen. In acutely exposed groups plenty of erythrocytes were also observed admixed with other cells.

During differential cell counts two rats of 4 weeks treated group showed presence of few bacilli in the field. In another rat of 8 weeks treated group clusters of bacterial colonies were found. This case also had a high neutrophil count of 50% and died just 5 days prior to completion of the tenure of 6 weeks inhalation. This rat was found to have florid bronchopneumonia with abscess formation and probably died due to pulmonary infection.

**Histopathology**

**Gross features:** Grossly the lungs looked normal in both the control and treated groups except in the quartz exposed rats where the lungs had a pale appearance.

**Microscopic findings:**

i. **Control groups (n=60):** On light microscopy all the control cases of both the acute and chronic models showed essentially normal lung parenchyma (Fig. 1). An occasional alveoli revealed presence of macrophages in the intra-alveolar space. Procedural haemorrhage was noted in a few cases. Only one control rat of 4 weeks inhalational group had consolidation of lungs.

ii. **Quartz treated intratracheal injection groups:**

Third day group (n=10)—Interstitial showed mild thickening and inflammation in two cases in other cases it was normal. Mild submucosal oedema with inflamed epithelium and few alveolar macrophages in the alveoli in four cases, and few dilated air spaces in one case were found. In two cases broncho-pneumonia were also observed. Three cases showed normal alveoli.

<table>
<thead>
<tr>
<th>Day after exposure</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>AM</td>
<td>89.50±1.3</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>2.40±0.3</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>8.10±1.1</td>
</tr>
<tr>
<td>5th</td>
<td>AM</td>
<td>91.00±1.1</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>2.40±0.3</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>6.00±0.9</td>
</tr>
<tr>
<td>7th</td>
<td>AM</td>
<td>87.10±0.9</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>3.10±0.5</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>9.80±0.5</td>
</tr>
</tbody>
</table>

All values are significant at $P<0.001$

**Table 3—Differential cell counts in BALF after inhalation of quartz**

<table>
<thead>
<tr>
<th>Duration of exposure</th>
<th>Cells</th>
<th>Cellularity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>AM*</td>
<td>89.60±1.9</td>
</tr>
<tr>
<td></td>
<td>N*</td>
<td>2.60±3.7</td>
</tr>
<tr>
<td></td>
<td>L***</td>
<td>8.30±1.8</td>
</tr>
<tr>
<td>4 weeks</td>
<td>AM*</td>
<td>84.50±1.0</td>
</tr>
<tr>
<td></td>
<td>N*</td>
<td>4.10±0.6</td>
</tr>
<tr>
<td></td>
<td>L*</td>
<td>11.40±1.0</td>
</tr>
<tr>
<td>8 weeks</td>
<td>AM*</td>
<td>86.70±1.6</td>
</tr>
<tr>
<td></td>
<td>N*</td>
<td>2.50±0.4</td>
</tr>
<tr>
<td></td>
<td>L**</td>
<td>10.80±1.4</td>
</tr>
</tbody>
</table>

P values: * $<0.001$; ** $<0.05$; *** $=$ Not significant

AM = Alveolar macrophage, N = Neutrophil, L = Lymphocyte
Fig. 1—Section of lung of a control rat showing normal lung architecture. H & E, × 80; Fig. 2—Section of lung of quartz exposed (7th day, intratracheal injection) rat showing peribronchiolar (P) and intraalveolar cellular infiltration (I) with foam cell (F) formation and dilated air spaces (D). H & E, × 200; Fig. 3—Section of lung of quartz exposed rat (2 weeks inhalation) showing formation of granuloma (G) with few foam cells (F) at the top. H & E, × 80; Fig. 4—Section of lung of quartz exposed rat showing focal collapse (c) of lung with few dilated air spaces (4 weeks inhalation). H & E, × 200; Fig. 5—Section of lung of quartz exposed (8 weeks inhalation) rat showing squamous metaplasia (M) with cellular infiltration in the alveoli. H & E, × 200.
Fifth day group (n=10)—Inflammation and focal thickening of interstitium were present in three cases and others showed normal, interstitium. Increased alveolar macrophages in six cases and few alveolar macrophages in other cases were found. Focal perivascular lymphomononuclear (neutrophil, eosinophil) cellular collections and submucosal oedema were present in three cases. Granuloma in one case with foam cells in two cases were also found. Dilated air spaces in two cases were observed.

Seventh day group (n=10)—Thickening and focal inflammation of interstitium with lymphomononuclear infiltrate were observed in two cases, others were normal. Neutrophilic emigration and increased macrophages with foam cells in the alveoli, peribronchial cellular infiltration, perivascular and bronchial sub-mucosal oedema, few dilated air spaces were found in six cases (Fig. 2). One case had epithelioid cell granuloma. Focal collapse was seen in two cases. One case was found with normal alveoli.

iii. Quartz exposed inhalation groups:

Two weeks group (n=10)—Thickening and focal inflammation of interstitium, increased alveolar macrophages with several foam cells, few dilated air spaces were observed in all the cases. Occasional giant cells in one case and dilated air spaces in five cases were also found. Three cases showed granulomas (Fig. 3).

Four weeks group (n=10)—Thickening of interstitium with less inflammation, and presence of inflammatory cellular infiltration with foam cells in the interstitium were observed in all cases. Focal collapse (Fig. 4) in three cases, denudation of bronchial alveoli in one case, and presence of profuse alveolar macrophages and foam cells, and occasional giant cells in the alveoli in all cases were found. Blood vessels were congested. One rat showed emphysematous changes and focal oedema. The severity of cellular infiltrate and thickening were much more increased than those of the 2 weeks groups. Three cases had granulomas.

Eight weeks group (n=10)—Thickening and inflammation with few foam cells in the interstitium were the usual pathological features in all the cases, which were more severe than those of the 4 weeks group. Focal haemorrhage with collapse, profuse alveolar macrophages with foam cells were the prominent features in all cases and the number of cells was much higher than those of the 4 weeks group. Granuloma in six rats and squamous metaplastic changes (Fig. 5) with bronchopneumonia in one rat were also observed.

Discussion

Intratracheal administration of quartz causes early and immediate biological changes and the effect simulates the effects of short and high dose of exposure to silica in human beings. The animals used in inhalation are surrogates for the humans. Inhalation is the only physiological route of administration of quartz that more realistically simulates the most probable human exposure. In the simulation chamber whole body exposure of the rats also mimics and generates the natural environmental exposure atmosphere of silica to the workers in the work field.

Gardener was one of the first investigators to draw attention to the nature of the cellular response in experimental silicosis. He described an influx of both neutrophils and macrophages with the predominance of the latter after silica deposited in the air spaces. The occurrence of cellular influx in lung tissue causing increased cellularity in BALF after silica exposure is well established. The significant increase in total cell counts in BALF found in this study supports the above findings. The rise was duration dependent in both the exposed groups. The cell counts increased with increasing duration of exposures.

Differential cell counts in BALF of both the quartz exposed models revealed duration dependent alterations in cellular pattern as compared to controls. There was relative decrease in percentage of alveolar macrophages with an increment in the absolute number in the exposed rats. Lymphocytes and neutrophils showed a concomitant and an important rise in the percentage. The initial cellular pattern was changed gradually in the later groups; the macrophages were being replaced by neutrophils and lymphocytes. So, the composition of cell types in BALF varied with duration and extent of exposures and this may be an indication of the severity of the disease. The findings of this study corroborate the observation of the study of Warheit et al showing increase in granulocyte count with the predominance of neutrophil following inhalation of quartz in rats. Similar change in cellular pattern with the exception of unchanged lymphocyte count of BALF after 1, 3, 14 and 28 days of intratracheal administration of quartz has also been reported by Gossart et al. The intratracheal injection of quartz in the previous and
the present experiments induced a fast cellular activation leading to the quick and sharp rise in the inflammatory cells.

The lung histopathological findings in quartz exposed groups of both the models showed significant alterations in normal lung architecture. The alveolar architecture was obliterated with cellular infiltration in the alveolar lumen. The cellular infiltration showed macrophages as the most prominent inflammatory cells with increased number of neutrophils, eosinophils, lymphocytes and foam cells confirming the pattern of inflammatory cellular infiltrates observed in the BALF. These changes were similar to those reported earlier in human silicosis\(^1\) and observed in experimental animals following inhalation and intratracheal administration of silica\(^9,15\). Neutrophilic emigration and oedema around blood vessels were significant findings in the intratracheal injection groups of the present study. Gossart \textit{et al}\(^1,18\) found a focal thickening of alveolar interstitium as early as first day posttreatment. In the present study interstitial thickening was observed on 3rd day of intratracheal exposure as the observations started on day three. The extent of the cellular infiltration and the thickening of interstitium were progressive and varied according to the durations of exposures of quartz. The inflammatory changes were more marked in inhalational model with higher density of cells. This persistent duration dependent pulmonary inflammatory response is consistent with the observations of progressive multifocal inflammatory lesions in short-term inhalation bioassay\(^7\).

In both the model the cellular infiltration led to formation of granulomas mainly consisting of histiocytes with scattered foci of lymphocytes. In the study of Gossart \textit{et al}\(^1,18\) the granuloma became apparent on day three following intratracheal administration of silica, whereas in the present study granuloma was observed on day five of intratracheal injection. However, these granulomas resembled early silicotic nodules. Multinucleated giant cells were occasionally seen around the granuloma and in the air spaces. The granulomas were organized with thin collagen bundles which is consistent with the findings of Hannotheiaux \textit{et al}\(^9\). The development of nodular and peribronchial fibrosis following inhalation and intratracheal injection of silica has been well documented and observed in several animal species with experimental silicosis\(^7,11-18\). However, none of the animals of this study showed silicotic nodules or fibrosis in the lungs. The maximum intensity of inflammation was seen in the \textit{w}rhinal region which is keeping with the fact that this site receives the highest concentration of silica.

In the present study squamous metaplastic change was observed after the prolonged inhalation of quartz. Squamous metaplasia in the 8 weeks inhalation group and granuloma in both the models were the most significant toxic changes of the silica exposure. Bronchopneumonia was seen in acute as well as in chronic models. This could be because of lung infection during the course of exposure. Emphysematous changes were also important findings and seen after prolonged exposure. It may have resulted from increased lung elastin degradation due to silica exposure. Similar emphysematous changes have been observed by Hannotheiaux \textit{et al}\(^9,13\) in silica exposed (inhalation) monkey's lung.

To conclude, the results showed quartz induced duration dependent cellular and histopathological changes which strongly correlate the increased toxic and progressive lung injury caused by silica. The significantly increased cellular changes in BALF along with inflammatory changes in lung histopathology on day three of intratracheal exposure of quartz could serve as early biological markers for silica induced lung injury. These early biological markers along with the measurement of silica content in the BALF may be used as valuable parameters for screening of the disease and as a diagnostic tool for silicosis much earlier than the roentgenographically detectable fibrotic changes in the occupationally exposed population. This early detection of the disease would help in employing some pharmacological interventions like antiinflammatory and antioxidant therapy along with other preventive measures, thereby preventing further damage to the lungs by silica.

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References


