Biomarkers of silicosis: Potential candidates

Abstract
Silica dust is widely prevalent in the atmosphere and more common than the other types of dust, thus making silicosis the most frequently occurring pneumoconiosis. In India also, studies carried out by National Institute of Occupational Health have shown high prevalence of silicosis in small factories and even in nonoccupational exposed subjects. The postero-anterior chest radiographs remain the key tool in diagnosing and assessing the extent and severity of interstitial lung disease. Although Computed Tomography detects finer anatomical structure than radiography it could not get popularity because of its cost. On the basis of histological features of silicosis many potential biomarkers such as Cytokines, Tumor Necrosis Factor, Interleukin 1, Angiotensin Converting Enzyme, Serum Copper, Fas ligand (FasL), etc. have been tried. However, further studies are needed to establish these potential biomarkers as true biomarker of silicosis.

Key words: Angiotensin converting enzyme, Biomarkers, Cytokines, Silicosis, Tumor necrosis factor

INTRODUCTION

Major proportion of the occupational diseases is formed by one of the ancient diseases known as pneumoconiosis. Since Ramazzini first described this group of respiratory disorders in coal workers,[1-3] numerous studies have been carried out on workers in various occupations exposed to various types of dust by virtue of their occupation. But the Silica dust is widely prevalent in the atmosphere and more common than the other types of dust, thus making silicosis the most frequently occurring pneumoconiosis.[1-3] Silica or silicon dioxide is formed from the elements silicon and oxygen under conditions of increased heat and pressure. It exists in the crystalline and amorphous forms. The most common form of crystalline silica is quartz, a typical component of rocks. Inhalation of various forms of free crystalline silica or silicon dioxide results in a spectrum of pulmonary diseases known as silicosis.

In India there are three million people, exposed to silica in mines and industries like stone cutting, silica milling, agate, slate pencil, etc. A substantial proportion of workers in construction activities like road building, also have potential exposure to silica. Studies carried out by National Institute of Occupational Health (NIOH) have shown high prevalence of silicosis in small factories[4,5] and even in nonoccupational exposed subjects in India.[6]

Histologically, silicosis is characterized by hyalinized and fibrotic nodules, thickening of alveolar interstitium, and accumulation of inflammatory cells such as alveolar macrophages (AM) and lymphocytes.[7] The pathogenesis of silicosis has been related to the accumulation of inflammatory cells that produce fibrogenic and inflammatory cytokines and growth factors, including tumor necrosis factor (TNF)-α,[8] interleukin (IL)-1,[9] transforming growth factor (TGF)-β,[10] macrophage inflammatory protein (MIP)-1 and MIP-2,[11] platelet derived growth factor, insulin like growth factor, and fibroblast growth factor.[12] An AM are thought to be key inflammatory cells in silicosis, since they produce most of these fibrogenic factors in silicotic lung.[10] The pro-inflammatory cytokine TNF-α plays a pivotal role in silicosis by mediating a wide spread inflammatory reaction and late fibrogenic reaction.[13,14]

The postero-anterior chest radiographs remain the key tool in diagnosing and assessing the extent and severity of interstitial lung disease. The International Classification of Radiographs of Pneumoconiosis published by International Labor Organization, is a widely accepted standard of radiograph for the classification of pneumoconiosis. However, concerns exist regarding the sensitivity and specificity of this diagnostic technique. For instance between 9.6
and 18.1% of individuals with pathological evidence of interstitial lung disease will have a normal chest radiograph. Moreover, the interpretation of standard chest radiographs using either descriptive terminology or the International Labor Office classification system has proved problematic in terms of inter and intra reader reliability. Similarly the lung function tests also reveal the changes in the advanced stages. Computed Tomography (CT) has recently been introduced for the diagnosis of pneumoconiosis. CT detects finer anatomical structure than radiography; it is expected to increase the sensitivity of diagnostic measures for this disease. However, there is a need to develop a biomarker for silicosis for early detection of silicosis. Thus the present review was done to find out the potential biomarkers of silicosis.

**Cytokines**

Alveolar macrophages play a key role in the development of silicosis by releasing a host of mediators, such as, cytokines and chemokines, which contribute to a complex network of interactions that result in the onset of lung injury, inflammation, and potentially fibrosis. Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by pulmonary macrophages and type II epithelial cells, and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells. In a murine study by Barrett et al cristobalite-induced MIP-2 mRNA levels were reduced by 52, 38, and 57%, with dimethyl sulfoxide, extracellular glutathione, or N-acetyl-L-cysteine treatment, respectively. Both MIP-1alpha and MIP-1beta mRNA levels were reduced at a magnitude similar to the reduction in TNF-α mRNA levels, whereas monocyte chemotactic protein (MCP)-1 mRNA levels were reduced at a magnitude similar to the reduction in MIP-2 mRNA levels following antioxidant treatment.

**Tumor necrosis factor**

Tumor necrotic factor (TNF) is a cytokine having two molecular species, TNF-α and TNF-β. The TNF-α induces the expression of a number of nuclear proto-oncogenes as well as other ILs. The TNF-β is characterized by its ability to kill a number of different cell types as well as the ability to induce terminal differentiation in others. The induction of TNF-β results from elevations of IL-2 as well as the interaction of antigen with T-cell receptors. Zhai et al found that compared with control subjects, increased TNF-α, IL-1beta, IL-8, and IL-6 levels were found in the bronchoalveolar lavage fluid BALFs in silicosis. However, Barrett et al reported decreased cristobalite-induced TNF-α mRNA levels in their murine study.

**Interleukin-1**

Interleukin-1 is also a cytokine. The IL-1 is a key mediator of the host response to various infectious inflammatory and immunologic challenges. The IL-1 alpha and IL-1 beta, mediate the biological activities and bind to the same cell surface receptors. Both are initially synthesized as 31 kDa precursors that are subsequently found as 17 kDa mature proteins. A large proportion of IL-1α has also been reported to be present on the cell surfaces. This membrane-bound IL-1α acts biologically in a paracrine fashion on those adjacent cells having IL-1 receptors. Intracellular IL-1 consists exclusively of the 31 kDa precursor from that shows little or no biological activity in comparison to the 17.5 kDa processed form. In human IL1 family consist of three genes located on long arm chromosome 2 that code for IL1-a, IL1-b, and IL 1 receptor antagonistic (RA).

**Angiotensin converting enzyme**

Lieberman in 1975 first reported the elevation of serum Angiotensin Converting Enzyme (ACE) in sarcoidosis. Several investigators have also confirmed that the serum ACE activity is increased in a large proportion of patients having granulomatous diseases like sarcoidosis and silicosis. Angiotensin 1-converting enzyme (ACE, peptidyl dipeptidase hydrolase, EC 3.4.15.1) is a membrane-bound glycoprotein, which converts Angiotensin 1 to Angiotensin 2 and participates in bradykinin degradation. The ACE is bound to the luminal membranes of endothelial cells, and its action takes place mainly in the pulmonary circulation. The serum activity of ACE in pulmonary diseases is of interest owing to its principal localization in the large capillary bed of the lungs.

**Serum copper**

One such possible biomarker could be serum Cu levels as it is reported in the literature that Cu has a fibrogenic property and as the primary pathologic changes in silicosis include fibrosis and the proliferation of collagen tissue in the lungs there could be possible association with raised levels of serum Cu. Although the mechanism of increase in serum Cu is still not understood, it has been suggested that an increase in ceruloplasmin levels in silicotics, which contains eight Cu atoms may be responsible for such an increase. Moreover, other studies have also reported elevated levels of serum Cu in silicotics. The serum copper levels as biomarker in those having exposure to silica dust without developing the disease is uncertain.

**Fas ligand (FasL)**

Silicosis is characterized by immunological abnormalities such as the appearance of autoantibodies and complications of autoimmune diseases. Dysregulation of apoptosis, particularly in the Fas/FasL pathway, has been considered to play a role in the pathogenesis of autoimmune diseases. The FasL is a membrane bound and shed protein belonging to the TNF gene family, and the natural counter-receptor for the death-promoting Fas molecule expressed by a variety of
lymphoid and nonlymphoid tissues.\textsuperscript{[20]} Lymphocyte apoptosis mediated by Fas/FasL interaction regulates immune responses\textsuperscript{[20]} and FasL-mediated apoptosis of leukocytes prevents inflammatory reactions at immune-privileged sites.\textsuperscript{[30]} Szczeklik \textit{et al.}\textsuperscript{[31]} carried out broncho-alveolar lavage in 11 patients of silicosis and found that in silicosis L-BAL apoptosis was inversely correlated with FEV1/VC values ($r = -0.26$, $P < 0.05$). Similarly Corsini \textit{et al.}\textsuperscript{[32]} in their experimental study among rats have also shown a decrease in FAS-L expression and silica-induced apoptosis in old macrophages. Hamzaoui \textit{et al.}\textsuperscript{[33]} examined the expression of Fas antigen, FasL and apoptosis in bronchoalveolar lavage fluid lymphocytes obtained from 10 patients with silicosis. They found Fas and FasL expression in silicosis patients to be significantly higher than those in healthy controls. In silicosis patients, FasL was highly expressed on CD4+, CD56+, and CD45RO+ bronchoalveolar lavage cells. They concluded that FasL was significantly expressed on cytotoxic effector and memory cells.

Thus to conclude, it can be stated that though many investigations have been tried to develop a suitable biomarker for silicosis, further studies are needed to establish a cost effective biomarker of the disease so that the early prediction of silicosis in exposed workers and its prevention can be effectively done.

\textbf{REFERENCES}

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