REVIEW ARTICLE

Merits and demerits of various biomarkers of chronic lung diseases with special reference to club cell protein 16 (CC-16) and early detection of chronic silicosis

Sumedha Mitra[1], Bidisa Sarkar[2], Kamalesh Sarkar[3]
[1]Prasanna School of Public Health, Manipal Academy of Higher Education, Manipal, Karnataka; [2]Department of Community Medicine, Gouri Devi Institute of Medical Sciences & Hospital, Durgapur, West Bengal; [3]Director, ICMR-National Institute of Occupational Health, Ahmedabad, Gujarat

ABSTRACT

Silicosis is a widely prevalent occupational lung disease with high morbidity and premature mortality. It is caused by continuous or intermittent exposure to respirable crystalline silica (RCS) dust while working in relevant work places. Conventionally silicosis is detected by the chest radiography and/or high resolution computerized tomography (HRCT) scan supported by evidence of impaired lung function test through spirometry. Secondary prevention of silicosis may be a possible strategy for reduction of silicosis associated morbidity and mortality provided there is a suitable biomarker available to predict it at its early stages among the silica dust exposed workers. This article has attempted to review the merits and demerits of various possible biomarkers such as silicon, respirable crystalline silica, TNF alpha, IL-6, IL-8, CC-16 etc. Of them, CC-16 has the distinct advantages over other markers. CC-16 is mostly secreted from the club cells of terminal bronchioles of lung and is easily diffusible in to the peripheral circulation. It is inversely correlated with the extent of silicotic lung damage. Recently Indian Council of Medical Research – National Institute of Occupational Health (ICMR-NIOH) through their research work has conclusively evidenced that CC-16 may be used as a proxy marker and screening tool for early detection of chronic silicosis by periodic screening among silica dust exposed workers. Further work towards CC-16 marker may be useful for control of chronic silicosis. This will also facilitate elimination of tuberculosis.

KEYWORDS

Occupational Lung Disease, Silicosis, Respirable Crystalline Silica (RCS), Biomarkers, CC-16

INTRODUCTION

Silicosis is a widely prevalent but grossly neglected occupational disease in India and in many other countries such as China, South Africa, Brazil, Australia, Thailand, Indonesia, the Philippines and some European countries. Silicosis is also an irreversible, incurable and progressive occupational lung disease due to continuous or intermittent exposure to respirable crystalline silica dust. Stone quarries, quartz mines, foundries, sand blasting and ceramics industries, gem cutting and polishing industries, slate/pencil industries, construction, glass and other mining industries are a few silica dust producing sectors that have been identified by the National Human Rights Commission, India [1]. India had an estimated 8.3 million workers, as of 1999, who were at risk of silicosis due to their day to day exposure while working [2]. This appears to be a gross underestimation due to methodological issues and today, the actual number must have at least doubled, if not more, owing to factors like growing population in the country, rapid urbanisation and industrialisation, presence of asymptomatic and/or mildly symptomatic early stages etc.; which are often undetectable by the conventional chest x-ray [3]. It may be noted that a large section of the working population of India is employed in the above-mentioned unorganised sectors, in which industrial hygiene and social security is almost non-existent. Absence of necessary dust control

CORRESPONDING AUTHOR: Kamalesh Sarkar, Director, ICMR-National Institute of Occupational Health, Ahmedabad, Gujarat
E Mail ID: kamalesh.sarkar@gmail.com
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measures in these largely resource poor settings; such as appropriate mechanised dust collection devices, water spraying enabled dust control systems, administrative control measures, providing adequate personal protective equipment; coupled with the reluctance to use them, have contributed to the rising number of silicosis within the vulnerable working populations in India [4].

Silicosis also exists in the non-occupational and/or para-occupational set ups. Small scale household industries like potteries engage workers and their family members in activities that produce respirable crystalline silica particles. [5]. Individuals who are not directly linked with these industries, but reside in places where a large number of such industries exist, or in which dust storms are frequent, are routinely exposed to silica dust and may develop silicosis upon long term exposure. In contrast to occupational forms of silicosis, women seem to be more frequently affected by non-occupational forms of silicosis. [6]

Silicotic patients are at lifelong high risk of pulmonary tuberculosis even after stoppage of silica dust exposure due to their progressively declining lung immunity [7,8] Inhaled silica particles trigger the secretion of inflammatory cytokines and initiate granuloma formation within the lungs by macrophages. This hinders the ability of macrophages to empty foreign matter, especially bacterial endotoxins including lipopolysaccharides [9], thus contributing to reduce lung immunity.

However, India is committed to elimination of tuberculosis by 2025 [10]. Considering the above, it appears that, unless silicosis is controlled, elimination of tuberculosis is not feasible. Primary preventive measures against silicosis continue to be increasingly difficult in a developing country like India due to a number of factors like: widespread poverty, lack of alternative work opportunities, lack of awareness, peer pressure, exploitative socioeconomic situations, resource poor work environments and increasing unemployment.

Silicosis, like many other occupational diseases in India, continues to lie in a “grey area” when it comes to authoritative responsibility. The Ministry of Health along with the Ministry of Labour, Mines & Industries are yet to formulate and implement a robust need-based policy that prevents occupational diseases. This stands out to be a need of the hour, especially because the workers involved in these industries are directly linked to the country’s productivity and economy. There is hardly any commitment or realistic silicosis prevention policy in most of the states in India. On the other hand, state authorities spend a large sum of money annually, to provide compensation to workers affected with silicosis [1]. Unfortunately, in most cases, the detection is too late, as the early stages of the disease are predominantly asymptomatic or mildly symptomatic and are easily overlooked. Moreover, early silicosis is not detectable by the conventional diagnostic methods like chest x-ray [11]; nor is there any other suitable operational mechanism to detect it in an early stage (subradiological silicosis).

In this light, secondary prevention of silicosis is a more realistic approach, provided early detection is possible through detection of a suitable biomarker which would indicate early silicosis, particularly if the vulnerable silica dust exposed workers are screened periodically.

The purpose of this literature review is to explore different biomarkers, direct or indirect, which can be potentially used for early detection of chronic lung diseases; especially silicosis, due to its widespread prevalence among the under-privileged workers with high morbidity/mortality.

MATERIAL & METHODS

A comprehensive search of online databases namely PubMed, Google Scholar and Scopus was conducted using the search terms “silicosis” or “biomarkers for chronic lung diseases”, or “CC16” or “biomarkers for pneumoconiosis”.

The search was limited to articles written in English and published since 1950. The reference lists of these articles were also searched and validated. The search was conducted in January, 2022. Apart from this, websites of regulatory bodies, both national, international and Indian government websites were also searched using the search term “silicosis and pneumoconiosis”.

RESULTS & DISCUSSION

A number of potential biomarkers for evaluating chronic lung diseases including extent of lung injury by the offending agent/s were reviewed. The purpose was to find out a possible marker that would indicate early silicotic lung damage among silica dust exposed workers on periodic screening. The ultimate purpose is to find a suitable biomarker for an effective public health intervention programme for prevention & control of silicosis. The reviewed bio-markers were

silicon, Krebs von den Lungen-6 (KL-6), matrix metalloproteinase-2 (MMP-2), surfactant protein D (SP-D), interleukin-8 (IL-8), platelet-derived growth factor (PDGF), 8-isoprostane, reactive oxygen species (ROS), glutathione, glutathione S-transferase, glutathione peroxidase (GPx), tumour necrosis factor-alpha (TNF-α) and club cell protein-16 (CC16). In the following sections, the relevant information gathered about these markers based on animal/human studies have been discussed.

Silicon: Hongli et.al. [12] used animal and human studies to explore whether silicon can be an important exposure marker in the development of silicosis. Serum levels of silicon were found to increase immediately in rats exposed to silicon dust. Similarly, their population study revealed that the silicon level in the silica exposed groups with and without obvious symptoms were significantly increased over that of the control group. In subjects with extended exposure to silica, the serum and urine silicon level in exposed workers appeared to increase rapidly, reaching its peak in 1-5 years, followed by a gradual decline thereafter. The workers with a total exposure time to silica dust of less than 10 years were further categorised into groups of 2 year intervals each. The levels of silicon in their urine and serum were found to be remarkably higher than that of the control group. The study concluded with the suggestion that determining the level of silicon in vivo might be an effective exposure marker in the diagnosis and pathogenesis of silicosis. But considering the fact that silicon level reaches a peak value between 1 to 5 year following exposure and then declines gradually, questions about its suitability for use in periodic screening.

Respirable crystalline silica: According to Elizabeth et. al. [13], mentioned about detection of respirable crystalline silica in the exhaled breath condensate (EBC) of sandstone quarry workers, may be used as a method for detection of silicosis in them. An EBC sample predominantly contains water vapour and epithelial lung lining fluid, and is usually collected through a mouthpiece. However, despite the non-invasive nature of collection and the utility of EBC as a biological “matrix” for monitoring occupational exposure to silica dust; it provides little information about the extent of lung damage and its correlation. Also, a need for standardisation of the collection and analysis procedure is also felt. This warrants the need for further in-depth studies to establish its viability as a sensitive and specific diagnostic/screening tool for evaluating progression of silicosis [14].

Other Markers: Xue et. al. [15] also identified other serum biomarkers for lung damage like Krebs von den Lungen-6 (KL-6), matrix metalloproteinase-2 (MMP-2), and surfactant protein D (SP-D); for diagnosis of pneumoconiosis like asbestosis and silicosis. In this study, the subjects belonged to either of the four groups: subjects with silicosis, subjects with asbestosis, dust exposed workers and healthy controls. KL-6, also known as MUC1 mucin in humans, has been used as a significant marker in diagnosis of various chronic lung fibroses. Secreted by type II pneumocytes in the alveoli of lungs, higher serum concentrations of KL-6 were linked with asbestosis, silicosis and idiopathic pulmonary fibrosis [15,16]. SP-D is one of the proteins present in the pulmonary surfactant that is also derived from type II pneumocytes. Patients with asbestosis and silicosis were found to have higher serum concentration of SP-D. However, SP-D was found to be a more effective marker for asbestosis than for silicosis. In case of MMP-2, although evidence exists that higher serum concentrations of MMP-2 are found in patients with idiopathic lung fibrosis, a strong correlation between serum MMP-2 concentration and pneumoconiosis like silicosis and asbestosis, was not established [15]. To summarise however, the authors recommended measurement of serum concentrations of all the three above-mentioned biomarkers for reliable results, and clarified that the dynamic changes in the serum concentrations of these markers could not be monitored in all the subjects [15]. Considering lack of requisite criteria for a near ideal screening/diagnostic test, there is need for further in-depth studies to understand the exact role of these biomarkers towards suitability for screening tests for silicosis and asbestosis.

Inflammatory biomarkers: Research has shown that serum inflammatory biomarker levels are elevated in silicotic patients, but the extent of their specificity for evaluating silicosis has not been well established. A review by Gulumian et. al. [17], mentioned that even today, the clinical detection of silicosis is largely dependent on chest X ray and lung function test anomalies, both of which are advanced manifestations of the disease. They conducted a robust review of literature on available biomarkers with the aim of identifying an ideal biological marker that is

capable of detecting silicosis and coal workers' pneumoconiosis (CWP) in its early stages. These included club cell protein-16 (CC16), interleukin-8 (IL-8), platelet-derived growth factor (PDGF), 8-isoprostane, reactive oxygen species (ROS), glutathione, glutathione S-transferase activity and glutathione peroxidase (GPx) activity. Oxidative stress is a major consequence of exposure to silica, and occurs by a number of mechanisms. Biomarkers of oxidative stress, like ROS, total antioxidant level, 8-isoprostane, glutathione, GPx and Glutathione S-transferase; all carry the potential to be used as a biomarker for the early detection of silicosis and CWP. However, the non-specificity of these markers to silicosis; especially due to the fact that mineral particles other than silica may also influence the levels of these markers, have led us to explore other biomarkers. Growth factors have also been known to be involved in fibrous lung diseases. PDGF secretion by alveolar macrophages was found to be stimulated by silica dust and coal dust. However, higher than normal serum PDGF levels were only detected in advanced silicosis patients, thus using it as a biomarker for early detection seems difficult. In conclusion, the authors opined that further studies were required to analyse the validity and practical feasibility of all the above mentioned biomarkers.

TNF-alpha: Slavov et. al. [18] conducted a study in Bulgaria, which explored the potential of tumour necrosis factor-alpha (TNF-α) as a biomarker for silicosis. They observed that TNF-α levels were similar in healthy individuals exposed to silica dust and silicosis patients. In contrast, the TNF-α levels of healthy individuals without prior exposure to silica dust were significantly lower. They also noted that there were no significant differences in the serum TNF-α levels in silicosis patients with moderate lung damage and severe lung damage. The need to correlate TNF-α levels with other biomarkers to indicate disease progression; coupled with the fact that TNF-α seemed to primarily indicate exposure to silica dust and not the extent of lung damage in silicosis patients, led us to question its feasibility as a reliable biomarker that could serve as a screening tool for silicosis.

Another longitudinal study by José et. al. [19] analysed numerous inflammatory biomarkers in silicotic patients. Manifestation of an inflammatory response to RCS, via an elevated number of lymphocytes, neutrophils and macrophages is a natural consequence. The study inferred about inflammatory markers, particularly interleukin 8 (IL-8) has the reasonable potential as a biomarker for assessing silicosis. However, it's role in the development and progression of silicosis was not well understood, and it seemed to be related to the development of progressive massive fibrosis. IL-8 is a chemotactic cytokine that can be produced by numerous tissues, including endothelial cells, macrophages and airway smooth muscle cells etc. [20]. Given its secretion by diverse tissues, it's specificity as a biomarker for silicosis or other pneumoconiosis remains uncertain.

Marker secreted predominantly from the lungs: Club cell protein 16 or CC16 appears to be an important marker towards this. CC16 (previously known as CC10), belongs to the secretoglobin family of proteins. Distal human lung epithelium is lined with abundant club cells, which account for as many as 22% of the cells in the lung epithelium [21]. Apart from club cells lining the human lung epithelium, CC16 is produced in small amounts by other tissues in the urogenital tract. However, the serum concentration of CC16 is found to be predominantly indicative of the amount secreted by the club cells lining the lung epithelium [22]. CC16 is highly soluble and diffuses easily into the circulation, where it can be measured. Acute exposures to endotoxin and wood smoke in healthy volunteers increases serum CC16 concentrations as early as 6 hr. after exposure, returning to normal within 24 hr [23]. Bernard A et al., observed that the highest concentrations of CC16 are observed in sputum and broncho-alveolar lavage fluid, reflecting intense secretion of the protein in the airways [24]. The protein is also found in smaller concentrations, in other fluids like amniotic fluid, urine and also, semen. Broeckaert and Bernard [25] found that Club cells are one of the most multifunctional and heterogeneous cell types in the mammalian lung, with their main function being protection of the respiratory tract. CC16 concentration in broncho-alveolar lavage fluid, which correlates well with its serum concentration, has also been shown to be a viable indicator of extent of lung damage in other lung diseases like chronic obstructive pulmonary disease (COPD) and asthma. [22,26] Serum concentration of CC16 was also found to be weakly yet significantly

correlated with declining lung function in COPD patients. This correlation was found to be stronger than that of other conventional inflammatory markers like IL-8 [27]. Lucky et. al. [28] mentions two mechanisms by which CC16 concentration decreases in silicotic patients. Firstly, the silica particles directly damage the Clara or Club cells, which are responsible for secretion of CC16. Secondly, these particles trigger an inflammatory response, and the Club cells are damaged by activated macrophages which release cytotoxic mediators. The authors, Jai Krishna Pandey & Deepa Agarwal reviewed the merits & demerits of various possible biomarkers of silicosis based on literature search & critical analysis [29]. They opined in their review paper that serum CC16 is a sensitive bio-marker and may be useful for silicosis detection compared to others.

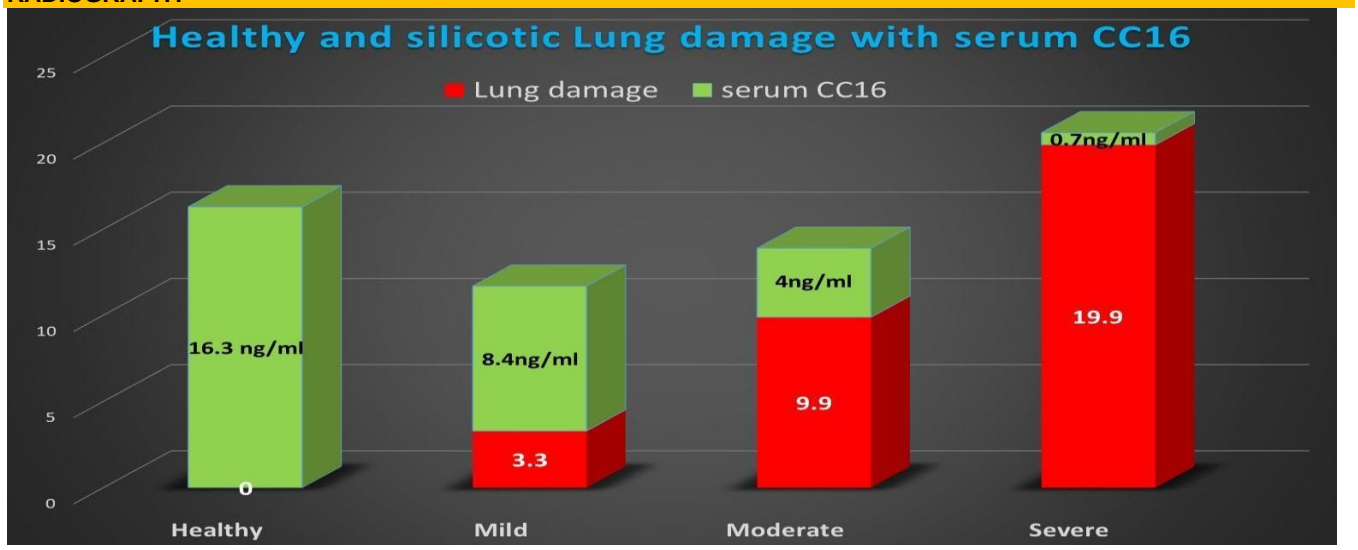
Bernard AM et. al. [30] measured the concentration of CC16 in the serum of 86 miners exposed to silica and 86 control subjects matched for age, body mass index and smoking status (each group had 60 current smokers and 26 lifelong smokers). Workers were exposed to silica-rich dust in a quarry for 15.2 months on an average. Pertaining to respiratory symptoms, chest radiographs or lung function tests; no appreciable difference was detected between the exposed and control workers. However, the serum CC16 concentration was markedly decreased in silica-exposed workers (with geometric mean 12.3 µg/l) compared to that of controls (16.3 µg/l). Tobacco smoking was found to reduce serum CC16 level, which was additionally lowered following exposure to silica. The investigators concluded that serum concentrations of CC16 probably reflect the very early toxic effects of silica particles on the respiratory epithelium. This reinforces the view that serum CC16 is a sensitive marker, which might improve the ability to detect exposure to chemicals potentially harmful to the respiratory tract.

A comprehensive review for biomarkers in silicosis and CWP by Gulumian, P. J. A. Borm, V. Vallyathan, V. Castranova, K. Donaldson, G. Nelson and J. Murray [17] reinforced the fact that CC16 could be used as a “reliable marker”, even for detecting the early stages of silicosis. An Australia based research group [14] also reviewed the available literature on silicosis and opined in favour of a bio-marker for early detection of silicosis. They also reviewed earlier work on CC16 [11] by ICMR-National Institute of Occupational Health, India, and opined CC16 as a potential early marker candidate for silicosis.

Screening for silicosis using CC-16: Recently Indian Council of Medical Research - National Institute of Occupational Health (ICMR-NIOH), has conclusively evidenced through their two research studies, that club cell protein 16 or CC16 may be a useful screening tool for early detection of silicosis among the workers with exposure to silica dust [11,31]. It may be noted that other research works on silica dust induced lung injury also supports the view of ICMR-NIOH’s work [29]. CC16, being a marker of chronic lung disease due to any cause, may be used as a proxy marker for early detection of any chronic lung disease including silicosis. When CC16 is selectively used as a screening tool for screening workers with a history of silica dust exposure, it appears to be an effective marker with high sensitivity and specificity. The ICMR-NIOH’s study [31] made a unique attempt to estimate and approximately quantify lung damage among silica dust exposed workers. This was done by using a novel scoring method of Lung Damage Score (LDS) using chest x-ray based opacities and involving ILO guidelines for assessing silicosis [32]. The study conclusively evidenced that serum CC16 levels are inversely related to the extent of lung damage - greater the lung damage, lower is the serum CC16 level (Figure -1).

Smoking has been found to reduce the serum CC16 levels additionally in both healthy as well as silica dust exposed workers, as revealed in the ICMR-NIOH study [11]. Hence, the workers need to be advised to refrain from smoking for 1-2 months prior to the screening, which would repair/regenerate the damaged cells and bring back CC16 level close to their pre-smoke level [11,31]. Alternatively, if refraining from smoking is not feasible, some adjustments to serum CC16 levels may be made. In case of heavy smokers (>10 cigarettes per day), 2 ng/ml and for moderate smokers (5 to <10 cigarettes per day), 1 ng/ml may be added to their serum CC16 level to adjust for the declined CC16 level due to smoking [11]. The inverse relationship of serum CC16 with progressive silica induced lung injury appears to be at least one step forward towards finding out a suitable biomarker for assessing chronic lung disease such as silicosis. This may be justified in absence of other suitable mechanisms for early detection of silicosis.

FIGURE-1: CORROBORATION OF SERUM CC-16 WITH SILICOTIC LUNG DAMAGE BASED ON CHEST RADIOGRAPHY



CONCLUSION

This review article attempts to understand the advantages as well as disadvantages of a number of possible biomarkers for screening and detection of chronic silicosis at its early stage for the purpose of secondary prevention of it. Most markers except club cell protein 16 (CC-16) may not be useful for assessing chronic silicosis due to their limitations mentioned already. CC-16 appears to be a reasonably effective proxy marker and screening tool. There is an inverse relationship between CC-16 level and different grades of silicotic lung damage among silica dust exposed workers as observed in ICMR studies.

AUTHORS CONTRIBUTION

SM and BS contributed equally by reviewing the literatures, analyzing the relevant information and initial drafting of the manuscript. KS guided the entire process and finalised the manuscript.

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