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Review

Inflammatory and oxidative stress parameters as potential early biomarkers for silicosis

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ABSTRACT

Workers involved in mining activities are exposed to crystalline silica, which leads to constant pulmonary inflammatory reactions and severe oxidative damage, resulting in silicosis. In this work, we aimed to evaluate inflammatory and oxidative stress parameters as potential early biomarkers of effect to assess crystalline silica toxicity in workers who had occupational exposure during mining. We enrolled 38 workers exposed to crystalline silica (WECS), 24 individuals with silicosis (IWS), and 30 occupationally unexposed workers (OUW), a total of 92 participants. The WECS were divided into 2 groups, according to the time of exposure: 19 workers with 1–15 years of occupational exposure (WECS I) and 19 workers with > 16 years of occupational exposure (WECS II). The inflammatory parameters assessed were L-selectin, β-2 integrin, and intercellular adhesion molecule-1 (ICAM-1) surface protein expression in lymphocytes and monocytes, complement C3 and C4, high sensitivity C-reactive protein (hsCRP), and adenosine deaminase (ADA) in serum. Plasma levels of malondialdehyde (MDA) and serum levels of vitamin C were determined as biomarkers of oxidative stress. Biochemical and hematological parameters were also investigated. L-selectin surface protein expression was significantly decreased in the WECS II group ($p < 0.05$), indicating the importance of this immune system component as a potential marker of crystalline-silica-induced toxicity. The MDA levels were significantly increased in the WECS I, WECS II, and IWS groups compared to the OUW group ($p < 0.05$). Vitamin C levels were decreased, while C3, hsCRP, ADA, and aspartate aminotransferase (AST) levels were increased in the IWS group compared to the OUW group ($p < 0.05$). Glucose and urea levels were significantly higher in the WECS I, II, and IWS groups compared to the OUW group ($p < 0.05$). Negative partial association was found between L-selectin and time of exposure ($p < 0.001$), supporting the relevance of this biomarker evaluation in long-term exposure to crystalline silica. Significant associations were also observed among inflammatory and oxidative stress biomarkers. Therefore, our results demonstrated the relevance of L-selectin as a potential peripheral biomarker for monitoring crystalline silica-induced toxicity in miners after chronic exposure, before silicosis has developed. However, more studies are necessary for better understanding of the use L-selectin as an early biomarker in exposed workers.

1. Introduction

Brazil is one of the most important exporters of gemstones in the world. Amethyst is among the most significant extracted gems, a violet variety of quartz, which is mostly found in the state of Rio Grande do Sul, Southern Brazil [1]. Amethyst mining is responsible for nearly 75% of all the economic activity in the town of Ametista do Sul, Rio Grande do Sul, Brazil, which has about 7000 residents and is the greatest producer of the *gem* worldwide [2, 3]. The town is located in a region that is part of the Serra Geral Formation, where amethyst-bearing basaltic lava flows have been identified [4].

Quartz is composed of crystalline silica (SiO_2), a compound that when fractured is harmful to the health of those who inhale the dust, especially mining workers. When respirable crystalline silica particles are inhaled, they are able to reach the alveoli, inducing oxidative stress by the formation of reactive oxygen (ROS) and nitrogen species (RNS) due to the generation of siloxil radicals after crystalline-silica fracturing [5]. Malondialdehyde (MDA), one of the better-known secondary

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products of lipid peroxidation, is a useful biomarker to evaluate oxidative stress. Moreover, the quantification of antioxidants, such as vitamin C, might help in oxidative stress evaluation [5–7].

Concomitantly, the particles unleash an inflammatory response that involves alveolar epithelial cells and elements of the immune system, for example, macrophages, neutrophils, lymphocytes, cellular adhesion molecules (CAMs), cytokines, chemokines, and complement system activation [5, 8, 9]. Important components of this reaction are L-selectin, a CAM (also known as CD62L) responsible for lymphocyte and neutrophil trafficking to inflammatory sites, and β-2 integrin and ICAM-1, CAMs involved in leukocyte migration to injured tissues. In addition, component C3 of the complement system is involved, which is a potent inflammatory mediator after activation. Adenosine deaminase (ADA), a ubiquitous enzyme present in inflammatory conditions and high-sensitivity C-Reactive Protein (hsCRP) can be altered during the inflammatory process as well [10–17]. Because they are present during early stages of the disease, they could be helpful for early diagnosis of silicosis.

Once the inflammatory response is not able to eliminate the silica particles from the alveoli, the reaction perpetrates in the lung tissue culminating in the generation of fibrotic nodules, characterizing silicosis. Silicosis in the most important occupational pneumoconiosis. Despite being known for so long, this disease is still responsible for the death of thousands of workers worldwide [5, 9, 10, 18–24].

This fibrotic process usually takes > 15 to 20 years to develop and depends on the amount of crystalline silica inhaled [20, 25]. Its main symptoms are cough, breathlessness, and weakness. Therefore, the disease is generally diagnosed by chest X-ray in late stages, when the lungs present considerable fibrotic nodules, and consequently, important loss of respiratory function. Because it is progressive, removing the worker from the workplace does not guarantee halting the progression of the disease. In addition, effective treatments for the problem are not available yet, and lung transplant is sometimes an option. Thus, individuals with silicosis become incapable of working and, as the disease progresses; they become unable to realize simple daily tasks [5, 20, 25–27].

In addition, crystalline silica is considered a carcinogenic compound belonging to Group 1 by the International Agency for Research on Cancer (IARC). Many in vitro and in vivo studies have demonstrated that crystalline silica exposure is linked to cancer development [28–33], especially due to its capacity to promote oxidative stress and chronic inflammation.

Therefore, considering the actual context of workers exposed to crystalline silica and the lack of an early way to diagnose silicosis, this study aimed to investigate inflammatory and oxidative stress parameters as potential early peripheral biomarkers of effect for monitoring workers with occupational exposure to crystalline silica in a mining town of Southern Brazil.

2. Material and methods

2.1. Participants

Four groups comprised this study, with 92 male participants, as demonstrated in Fig. 1. The group of workers who were not occupationally exposed (OUW) consisted of 30 men who worked in administrative functions in the city of Porto Alegre, Rio Grande do Sul, Brazil, and never had any history of crystalline silica occupational exposure. The group of workers exposed to crystalline silica (WECS) consisted of 38 men who worked in mining activities in the town of Ametista do Sul, Rio Grande do Sul, Brazil (Fig. 1), with no diagnosis of silicosis.

Because there were workers with different times of exposure, we divided the WECS in 2 groups: WECS I, composed of 19 workers with 1 to 15 years of occupational exposure, and WECS II, composed of 19 workers with 16 or more years of occupational exposure. All miners worked in underground mines. The last group consisted of 24 individuals with silicosis (IWS), who had been diagnosed with silicosis and are retired from mining activities.

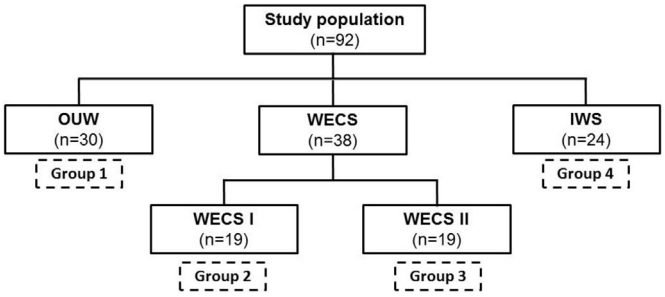

Fig. 1. Schematic demonstration of the division of groups from the study population. Abbreviations: OUW: occupationally unexposed workers; WECS: workers exposed to crystalline silica; WECS I: workers exposed to crystalline silica with 1–15 years of occupational exposure; WECS II: workers exposed to crystalline silica with > 16 years of occupational exposure; IWS: individuals with silicosis.

Recruitment of exposed workers and IWS was achieved by contact with a public facility in the town especially focused on miner's occupational health. Nurses and the doctor of the facility contacted the mining companies, the workers, and the IWS and invited them to participate in the study.

Individuals with chronic diseases (diabetes, hepatitis, tuberculosis, or previous cancer history) and those with < 1 year of occupational exposure to crystalline silica were excluded from the study.

All the participants answered a questionnaire regarding health status, lifestyle, drinking, smoking, and exercising habits, diet, use of medicines, and occupational activities. This study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande do Sul (Registry CAAE n° 60,976,516.7.0000.5347). All participants were informed about the study and signed an informed consent.

2.2. Sample collection

Blood of all participants was collected in the morning, after an 8-hour fasting period, by venipuncture using vacutainer tubes. An EDTA tube (4 mL) was used for the blood count and L-selectin, β-2 integrin, and ICAM-1 surface protein expression. The EDTA-blood was centrifuged at 1500*g* for 10 min at room temperature to quantify malondialdehyde (MDA) in plasma. An anticoagulant free tube (5 mL) was also centrifuged at 1500*g* for 10 min at room temperature, and the serum was used to perform ADA activity determination and the following biochemical analyses: glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine, and serum total protein determinations. The remaining serum was stored at −20 °C for later analysis of the components 3 and 4 of the complement system (C3 and C4, respectively) and high sensitivity C-reactive protein (hsCRP); serum was stored at −80 °C for vitamin C analysis. In addition, 50 mL of urine was collected from each participant to determine creatinine and microalbuminuria levels, and the remaining urine was frozen at −80 °C for further analysis.

2.3. Anthropometric measurements

On the same day of blood and urine collection, participants were evaluated for weight and height to obtain the body mass index (BMI). Weight was measured with an upright scale (Plenna Sport MEA 07420, São Paulo, SP, Brazil), with a capacity to weigh 150 kg in 100 g increments. Height was measured using a standing stadiometer. BMI was obtained by division of the person's weight in kilograms (kg) by the square height in meters (m) (kg m^2).