Lactate dehydrogenase as a biomarker for silica exposure-induced toxicity in agate workers

Bhagwan Das Aggarwal

ABSTRACT

Objectives Agate workers are chronically exposed to silica dust generated from agate grinding, which makes them susceptible to silicosis. In the absence of diagnosis at an early stage, the workers continue to be exposed to silica dust until the development of silicosis. The present study was undertaken to investigate total lactate dehydrogenase (LDH) activity in blood samples of silica-exposed agate workers as a non-invasive way to measure silica-induced toxicity.

Methods Blood samples were collected from agate workers and control subjects. Total LDH activity was measured in the blood plasma and blood cells of agate workers and non-exposed (control) subjects using sodium pyruvate as a substrate. The reduction of pyruvate to L-lactate with the concurrent oxidation of nicotinamide adenine dinucleotide (NADH) during the assay was monitored by change in absorbance (or optical density (OD)) at 340 nm at the fixed interval of 10 min. The ratio of LDH activity (blood plasma/blood cells) in the blood samples was calculated as a measure to detect cytotoxicity in exposed workers.

Results The LDH activity in blood plasma samples of exposed workers was found to increase about 25 times, while the activity in the blood cells of silica-exposed agate workers was reduced to 10% of control subjects. The ratio of LDH activity (blood plasma/cells) was found to be 6.6 in the silica-exposed agate workers, while it was 0.02 in control (non-exposed) subjects.

Conclusions This study proposes that total LDH activity and the LDH ratio (plasma/cells), along with occupational exposure history, are markers for silica exposure-induced toxicity in agate workers.

INTRODUCTION

The lactate dehydrogenase (LDH) enzyme is an intracellular enzyme in all body cells, including blood cells, but only a small amount is normally detectable in blood plasma. The enzyme levels in various tissues are very high compared with the level in blood plasma. Earlier reports suggest that the LDH activity in tissues could be 500 times higher than the LDH activity normally found in blood plasma. Any leakage of LDH from damaged cells can significantly increase the LDH activity in plasma. During anaerobic glycolysis, LDH catalyses the interconversion of pyruvate into lactate. The extracellular appearance of LDH signifies cell damage and a host of disorders.

Studies in rats have shown that silica exposure induces cell damage and causes a 10-fold increase of LDH in bronchoalveolar lavage (BAL) fluid within 24 h, and an increase of 20–60 times after 20–60 days of the exposure. Hayashi et al showed that 24 h of silica exposure in rats could induce a 10-fold increase of LDH in blood serum. A study in coals miners suggests that coal dust exposure increases LDH activity in serum even after stoppage of exposure of coal dust. Most of the previous LDH studies in vivo, suggested increases of LDH activity in serum and BAL fluid of silica-exposed animals. However, not many studies are available for LDH activity in silica-exposed human subjects. This study is the first to investigate levels of LDH activity in blood cells as well as in blood plasma for silica-exposed agate workers, and it also suggests the ratio of LDH activity (blood plasma vs blood cells) as a marker for silica exposure-induced cytotoxicity in exposed subjects.

Agate workers in the Khambhat region of India are involved in cutting, grinding and polishing of agate stones. Most of the agate workers are uneducated and financially poor, and they do not use any dust protective methods. Agate is a semiprecious stone and contains crystalline silica, and the process of cutting and grinding is used for ornamental and jewellery purposes. Agate stone is ground on a wheel driven at speeds of 2000–3000 rpm. The grinding process generates large amounts of respirable (2–5 microns size) silica dust. The dust contains 60–90% of free silica. Respirable dust...
concentrations in the indoors of an agate household unit is typically about 15 times higher (2.35–7.4 mg/m³) than the permissible level of 0.16 mg/m³. Inhalation of silica dust (SiO₂) causes many hazardous health effects worldwide, including cancer.  

Exposure assessment

The analytical chemical reagents, viz nicotinamide adenine dinucleotide – reduced (NADH), sodium pyruvate, bovine serum albumin, Tween-20, Triton X-100, Tris HCl, Tris Base, EDTA, Coomassie Brilliant Blue G-250 and bromophenol blue were obtained from Sigma Chemical, USA. The KCl, MgCl₂, phosphate buffer saline tablets, NaCl and sodium acetate were obtained from Qualigen, India; and the analytical grade methanol, butanol and glacial acetic acid were procured from SRL, India.

Materials and methods

Chemicals

The objectives and methodology were explained to the subjects in their local language(s). Information about the source of silica dust exposure, duration of exposure and heath status was obtained from a 20–30 min personal interview with each worker. The control samples were collected from a local blood bank (Civil Hospital, Ahmedabad) and from local healthy (non-exposed) donors.

Exclusion criteria

Subjects with chronic health conditions like diabetes or cardiovascular diseases were excluded from the test (silica exposed agate workers) and the control groups. In the control group, subjects having pulmonary tuberculosis or chronic cough were also excluded.

Lactate dehydrogenase assay

The blood plasma and blood cells were separated from the blood samples by centrifugation at 2000 rpm for 20 min. The cells were lysed using the lysis buffer (10 mM Tris base, 10 mM KCl, 10 mM MgCl₂, pH-7.6), and the cell lysates were cleared of any cell debris by centrifugation at 12,000 rpm for 15 min. LDH activity was measured in the blood plasma and cell lysates at room temperature using sodium pyruvate as a substrate. In brief, 10 μL of blood plasma or 10 μL of cell lysate was incubated with 3.0 mL of assay substrate (0.2 M Tris HCl, 6.6 mM NADH, 30 mM sodium pyruvate) for 10 min. The reduction of pyruvate to L-lactate with the concurrent oxidation of nicotinamide adenine dinucleotide (NADH; or reduced form) during the assay reaction was monitored by change in absorbance (or change in optical density – ΔOD) at 340 nm by UV-Visible Spectrophotometer V-560 (JASCO, USA) at fixed intervals of 10 min. The ΔOD at 340 nm is caused by the oxidation of NADH, which is directly proportional to the LDH activity. One unit of LDH causes the oxidation of one μmole of NADH per minute under specified conditions.

LDH activity was calculated using the following formula:

$$\text{LDH activity Unit} = \text{ΔOD}_{340} \times \text{F} \times \text{Sample Volume (mL)/(6.22 \times \text{Assay Volume (mL)}})$$

Sample collection

Blood samples were collected from 35 silica-exposed agate workers and 27 non-exposed (control) subjects. The blood samples were collected in EDTA vacutainers after obtaining written informed consent of agate workers (subjects) from three villages (Metpur, Timba and Sakarpura) of the Khambhat region (Gujarat, India). Local healthcare workers (known as ‘Asha’ workers) assisted in identifying the agate workers. The research

Study and ethical issues

The Institutional Review Committee and Ethics Committee of the National Institute of Occupational Health, Ahmedabad, reviewed the study and provided ethical approval for collecting blood samples from the agate workers after their informed consent.

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RESULTS

Profile of non-exposed (control) and silica-exposed agate workers
The mean age of control subjects was 31±9 years, and it was 42 ±11 years for silica-exposed agate workers. Out of 35 agate workers (exposed group), only one worker was a smoker and 17 workers were tobacco chewers. Most of the workers worked 6–8 h every day, and 95% (33) of the agate workers had worked in the agate industry for an average of 16±8 years. The exposed group consisted of 14 female and 21 male agate workers, but the control group was all male (table 1).

The control samples were not collected from non-agate workers/subjects from the same villages but were collected from a blood bank (Civil Hospital, Ahmedabad) and from healthy (non-exposed) donors because the non-agate subjects living in those villages were also exposed to persistent environmental silica dust generated from local agate grinding units. The data suggest that environmental dust concentration in nearby villages varied from 7.06 mg/m³ to 28.15 mg/m³ with the average of about 15.28 mg/m³, which is about three times more than the Environmental Protection Agency’s (http://www.epa.gov) recommended air quality standards of 5.0 mg/m³.15

Effect of silica exposure on LDH activity in blood cells of agate workers
Most of the earlier studies estimated the LDH activity in blood serum or BAL fluid of dust-exposed workers but not in blood cells of the exposed. Blood cells have a significant amount of LDH enzyme, and silica exposure could be directly or indirectly modulating the LDH activity in blood cells. In this study, we have for the first time estimated the status of LDH activity in the blood cells of silica-exposed agate workers and control subjects. Figure 1 represents LDH activity in the blood cells of 19 silica-exposed agate workers and control subjects. Figure 1 and table 2). The lower level of LDH activity in plasma of silica-exposed agate workers, with more extracellular LDH enzyme being released into the blood plasma.

Effect of tobacco usage on LDH activity
Among 35 agate workers, one worker was a smoker and 17 were tobacco chewers. LDH activity was measured in the blood cells and blood plasma of tobacco users as well as non-tobacco users (agate workers) to check if tobacco usage has any effect on LDH activity (figure 3). The results suggest that there is no significant effect of tobacco usage on LDH activity in blood cells (figure 3A) or blood plasma (figure 3B) of the silica-exposed agate workers.

Table 1 General profile and details of control subjects (unexposed) and silica-exposed agate workers in Kambhata (Anand district, Gujarat, India)

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Exposed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>Male (n)</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Female (n)</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Silica exposure, in years</td>
<td>0</td>
<td>16±8</td>
</tr>
<tr>
<td>Mean age, in years</td>
<td>31±9</td>
<td>42±11</td>
</tr>
<tr>
<td>Smokers</td>
<td>n. a.</td>
<td>1</td>
</tr>
<tr>
<td>Tobacco chewers</td>
<td>n. a.</td>
<td>17</td>
</tr>
</tbody>
</table>

Effects of gender on LDH activity
The exposed group consisted of 14 female and 21 male agate workers, but the control group was all male. Gender could be affecting LDH activity in the exposed group of agate workers; hence, LDH activity was measured in male and female agate workers and compared (see online supplementary figure S1). The data suggest that there is no statistically significant difference in LDH activity in the blood cells (see online supplementary figure S1a) or the blood plasma (see online supplementary figure S1b) of either male or female agate workers. Therefore, gender may not be a significant factor in the LDH activity of agate workers.

Effects of silica exposure on LDH activity in blood plasma of agate workers
The high level of circulating extracellular LDH in blood plasma suggests cellular damage. Therefore, the LDH activity in the blood plasma was measured and compared for silica-exposed agate workers and non-exposed (control) subjects. Figure 2 compares the blood plasma LDH activity of 23 exposed workers and 17 non-exposed (control) subjects (after removing invalid (negative) values). Analysis of the data suggests that the LDH activity is about 25 times higher in the plasma of the exposed workers than the non-exposed (control) subjects (figure 2, table 2). The higher level of LDH activity in plasma samples of the exposed group suggests that silica exposure might have induced cellular and tissue injuries in the silica-exposed agate workers, with more extracellular LDH enzyme being released into the blood plasma.

Figure 1 Lactate dehydrogenase (LDH) activity in the blood cells of control subjects (n=15, mean age 31±11 years) and silica-exposed agate workers (n=19, mean age 42±12 years). Although the LDH activity was measured in all exposed and control subjects, invalid (negative) values were removed and not considered. Here mean age of subjects represents valid values only. Each point represents LDH activity from each individual subject. LDH activity is expressed in unit per mg of protein. Values represented as mean±SEM. An asterisk (*) indicates statistically significant difference over controls (p<0.0001).

Figure 2 Comparison of the blood plasma LDH activity of 23 exposed workers (mean age 42±12 years) and 17 non-exposed (control) subjects (mean age 31±11 years). The analysis suggests the LDH activity is about 25 times higher in the plasma of the exposed workers than the non-exposed (control) subjects. An asterisk (*) indicates statistically significant difference over controls (p<0.0001).
Table 2  Lactate dehydrogenase (LDH) activity and the LDH ratio (plasma/cell) in control subjects (unexposed) and silica-exposed agate workers

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Exposed*</th>
<th>Fold difference controls vs exposed (p Value, t test)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood cells</td>
<td>0.0586±0.0065 (n=15)</td>
<td>0.0047±0.0003 (n=19)</td>
<td>12.5 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Plasma (serum)</td>
<td>0.0013±0.0001 (n=17)</td>
<td>0.0313±0.0032 (n=23)</td>
<td>0.04 (p&lt;0.0001)</td>
</tr>
<tr>
<td>Ratio (plasma/cell)</td>
<td>0.022</td>
<td>6.66</td>
<td></td>
</tr>
</tbody>
</table>

*LDH activity—unit/mg of protein±SEM.
†Unpaired two-tailed student t test p<0.0001, difference is statistically significant at p<0.05 (by GraphPad PRISM software).

Cell lysis in plasma of agate workers

The blood samples from the agate workers were collected and transported to the laboratory under cold conditions (4–8°C) for further analysis, while the control samples were collected from a local blood bank and transported under cold conditions (4–8°C) to the laboratory in less than an hour. The blood samples from the exposed group were under transit for 4–6 h and might have undergone some blood cell lysis, which could be a reason for the higher LDH activity in the blood plasma of the exposed workers. The amount of blood cell lysis in the control and exposed plasma samples, which were used for the LDH assay, was detected by measuring the OD at 415 nm (OD of haemoglobin) as shown in online supplementary figure S2. The OD data are comparable for both groups and suggest that there was no significant blood cell lysis in exposed blood samples during the transport or storage of samples, and may not have contributed to the higher LDH activity in the plasma samples of the exposed workers.

Ratio of LDH activity in blood plasma versus blood cells of the control group and the exposed group

The data in table 2 suggest that, for the control subjects, the LDH activity in the blood plasma was about 0.02 times the LDH activity in the blood cells (LDH ratio plasma/cells=0.02), which is consistent with our knowledge that LDH is a cellular enzyme and is mostly present in the cytoplasm of cells. From the study data, only a fraction (about 2%) of blood cell LDH activity was found in the blood plasma of the control subjects. In silica-exposed agate workers, the LDH activity was about seven times higher in the blood plasma than in the blood cells (LDH ratio of blood plasma/cells=6.66). This result indicates silica exposure might have caused, either directly or indirectly, damage to the blood cells of silica-exposed workers resulting in the decrease of LDH activity in blood cells. For the first time, this study compares and reports the ratio of LDH activity in blood plasma compared with blood cells in the control and exposed groups.

DISCUSSION

Extracellular presence of LDH in serum or plasma could indicate exposure or disease-induced cellular damage.1-5 Earlier reports have proposed an increase in blood serum LDH activity as a biomarker of tissue and cellular injuries and as a useful marker for monitoring disease condition.1-2 4 11 Silica exposure could induce pulmonary nitric oxide and reactive oxygen species production, resulting in severe oxidative stress causing pulmonary inflammation and cell damage, which could lead to lung fibrosis and silicosis.5-6 Studies have shown that silica-exposure induced lung damage could increase extracellular LDH in BAL fluids in animal models.5-6 9-11 Limited LDH data are available for BAL fluid, or serum or in plasma of silica-exposed human subjects. One study has shown increased
LDH activity in the lung lavage (BAL) of Québec granite workers. In another study of serum samples from exposed subjects, Cobben et al reported a significant increase (about four times) in LDH activity in ex coal miners, and a recent study by Deniz et al suggested significantly high serum LDH levels in patients with silicosis.

High LDH levels in plasma samples of the exposed subjects in this study are similar to the previous studies of LDH activity in the serum of ex coal miners and patients with silicosis. The agate workers in this study were exposed to 15 times higher than permissible levels of silica dust, for 6–8 h every day for an average of 16.8±2 years. This long-term chronic exposure to silica dust might have induced lung injuries resulting in a significant release of LDH and other cellular enzymes into the bloodstream resulting in higher LDH activity in plasma samples of the silica-exposed agate workers.

The higher level of circulating LDH in the plasma of the exposed workers might have further induced cellular injuries in blood cells, resulting in further release of LDH from the blood cells into the plasma. This could be a reason for the significant decrease of LDH activity in the blood cells and the significant increase of LDH activity in the blood plasma of the silica-exposed agate workers. Since, most of the workers in this study were exposed to silica dust for an average of 16.8±2 years, a similar study should be conducted for workers who were exposed for less than a year to determine if blood LDH could be used for early silica exposure-induced cell damage. Considering the significant difference in LDH activity in exposed workers, this study proposes that total LDH activity in plasma samples, along with worker’s occupational history, could be used as a marker for silica-induced cellular injury in agate workers.

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Contributors BDA has designed and executed the study with the assistance of technical staff, who are properly acknowledged in the manuscript. BDA performed the analysis and interpretation of the data and wrote the manuscript. The funding agency has no role in planning, execution or interpretation of the data or outcomes.

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Competing interests None.

Patient consent Obtained.

Ethics approval Institutional Review Committee and Ethic Committee—the ‘IRB’ of National Institute of Occupational Health, Ahmedabad, India.

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