Effect of Occupational Silica Exposure on Kidney with Emphasis on Urinary Amino Acids as Indicator for Tubular Dysfunction.

Safia B Ahmed, Khadiga S Ibrahim, Hisham M Aziz and Weam A Shaheen

Environmental and Occupational Medicine Department, National Research Center, Egypt.

ABSTRACT

The aim of the current study was to investigate the association between occupational silica exposure and renal dysfunction. The study included 21 silicotic and 26 non-silicotic workers from cement industry and 25 male with no history of occupational exposure to silica or solvents (as a control group). All subjects filled a questionnaire sheet and underwent clinical examinations. Urinary level of microalbumin, α1-microglobulin, NAG, zinc, copper, and analysis of 17 amino-acids were done for all subjects. A high significant increase in the urinary excretion of amino acids and all measured urinary parameters in the group of silica-exposed workers were found, compared with the control subjects. The occurrence of amino aciduria would be an important investigation for the toxic effects of the silica exposure.

Key words: Silica Exposure, Microalbuminuria, Aminoaciduria, Renal dysfunction, Pneumoconiosis.

Introduction

People are exposed to various potentially toxic agents in their natural and occupational environments. These agents may be physical or chemical, may enter the human body through oral, inhalational, or trans-dermal routes, and may exert effects on all systems (Soderland et al., 2010).

Silica, a chemical compound found in abundance in nature, is comprised of quartz, a constituent of rock, and makes up 90-95% of sand. Most silica in commercial use is obtained by processing (such as crushing or milling) from naturally occurring sources. The chemical compound silicon dioxide, also known as crystalline free silica, is an oxide of silicon with a chemical formula of SiO₂ and has been known for its hardness since antiquity. Silica exists in the crystalline and amorphous forms (Calvert and Steenland, 1997).

Industries and occupations having the potential for silica exposure include mining, quarrying, tunneling, foundry work, glass manufacture, abrasive blasting, ceramic and pottery production, and cement production (Cvariani et al., 2005).

The epidemiologic data are conflicting on the mechanism by which silica causes kidney disease. The mechanisms by which silica may damage the renal system can be either through direct (silica particles in the kidney) or indirect toxicity (Steenland et al., 2002). This indirect toxicity likely occurs when the lungs, after being exposed to silica particles, begin to produce macrophages to attack the particles. This process, in addition to lymph node stimulation, activates the immune system and can lead to glomerulonephritis (Steenland and Goldsmith, 1995). Renal disease without pulmonary changes has been associated with silica exposure and may manifest as nephritic syndrome, glomerulonephritis or renal failure (Rosenman et al., 2000).

Renal urinary biomarkers that proved most useful to define effects on various parts of the nephron include the high-molecular-weight protein albumin for evaluating glomerular integrity; the low-molecular-weight protein α1-microglobulin for assessing tubular reabsorption function (α1M); the lysosomal enzyme N-acetyl-d-glucosaminidase (NAG) to indicate tubular injury (Finn and Porter, 2008). Increased urinary excretion of zinc and copper might be a reflection of a disturbance in the homeostasis of these essential elements that might, in turn, contribute to a mechanism of nephrotoxicity (Ibrahim et al., 2011). The renal excretion of individual and grouped amino acids is an indicator of decreased tubular re-absorption of low-molecular weight proteins (Lauwerys and Bernard, 1987).

Aim of the Work:

The aim of the current study was to investigate the association between occupational silica exposure and renal dysfunction, to study the difference in renal excretion of amino acids profile and to determine if any amino acid transport systems were impaired.
Materials and Methods

Subjects:

This work was a cross-sectional controlled study. The study was conducted at a cement industry located in Helwan district, Cairo Governorate, Egypt.

Inclusion and Exclusion Criteria:

In both the exposed and control groups, subjects with past medical history of hypertension, diabetes mellitus, evidence of chronic renal diseases (gout, urinary tract infections, glomerulonephritis or renal calculi) and on regular consumption of drugs with potential nephrotoxicity as (antibiotics, analgesics and steroids), or workers with a previous or current potential exposure to agents known to be nephrotoxic, such as heavy metals or solvents were excluded from this work. After application of the aforementioned criteria, the studied population consisted of the following:

Twenty-one silicotic workers from different departments of the cement industry (quarrying, crushing, milling and packing processes) having a mean duration of exposure 27.30±4.43 years. None of them used any personal protective equipments as masks or respirators during their job. Those cement workers were previously diagnosed as silicotics by the Occupational Diseases Committee, Health Insurance Agency, Cairo and they had occupational disability ratio (ranged from 5 to 25%) and their chest X-rays (confirmed by High Resolution Computerized Tomography scan) revealed that all the opacities were small and round in the form of p & q categories and profusion categories were 1/0, 1/1, 1/2 and 2/1.

Twenty-six non-silicotic workers randomly chosen from different departments of the cement factory and had a mean duration of exposure 26.77±5.57 years also without using any personal protective equipments as masks or respirators during their job. Their chest X-rays and CT scan examinations revealed that all of them were free of silicosis.

Twenty-five male clerks (as a control group) randomly chosen from National Research Center with no past or current history of occupational exposure to silica or solvents and with no history of primary or secondary renal diseases.

All the exposed workers and the controls were non-smokers males with matched ages (mean age was 53.10±4.46 years for the silicotic group and 50.92 ±5.02 years for the non-silicotic group vs. 52.85 ±3.86 years for the controls) and of the same socio-economic standards.

Methods:

1- Written informed consent was taken from each subject for his voluntary participation in this study and signed by the principal investigator and the participant. All subjects were interviewed to complete questionnaire sheet (personal data, smoking habit, detailed current and previous occupational history to find out work-related symptoms and past medical history of chronic illness or drug abuse).

2- For all subjects, general clinical examination was done including measuring of the blood pressure using mercury sphygmomanometer and detection of glucose in urine using a strip test to exclude hypertensive and diabetics. Local chest and abdominal examination were done.

3- Plain chest X-ray: Full sized (14” × 14” inches) roentgenograms posterior-anterior view, and High Resolution CT scan were performed for the exposed workers, and the roentgenographic findings were classified according to the I.L.O classification of pneumoconiosis (International Labor Office, 2002).

4- Laboratory Investigations:

A random morning urine sample was collected from each participant in acid washed plastic container and centrifuged at 4500 rpm for 10 min; then the top 15 ml of the supernatant was stored frozen at −20°C in aliquots without preservatives until analysis of the following:

a- Urinary level of microalbumin (U-Malb) by colorimetric assay (Watanable et al., 1986) using kit from Spectrum Diagnostics, Egypt.

b- Urinary α1-microglobulin level by means of ELISA using kit from Arbeitsanleitung (Bensheim, Germany).

c- Urinary activity of NAG (U.NAG) by colorimetric assay (Price and Wbiting, 1992) using kit from Diazyme (USA).

d- Urine concentration of copper (U.Cu) by colorimetric assay (Abe et al., 1989) using kit from LTA Milano, Italy.

e- Urine concentration of zinc (U.Zn) by colorimetric assay with 5-Brom-PAPS using kit from Greiner Diagnostic GmbH-Germany according to Fuentes et al. (1982).
f- Urine concentrations of silica according to Paul (1960).

g- The analysis of urinary amino acids was performed in Central Service Unit, National Research Center, Egypt using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany) according to Spackman et al., 1958. The technique was based on the separation of the amino acids using strong cation exchange chromatography followed by the ninhydrincolour reaction and photocrometric detection at 570nm. Samples were hydrolyzed with 6N HCl at 110°C in Teflon capped vials for 24h. After vacuum removal of HCl, the residues were dissolved in a lithium citrate buffer, pH 2.2. Twenty µl of the solution were loaded onto the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at flow rate 0.2 ml/min. The ninhydrin flow rate was 0.2 ml/min and pressure of 0-150 bar. The pressure of buffer was from 0 to 50 bar; and reaction temperature 130°C.

h- Urine creatinine (U.cr) concentration by colorimetric method (Bowers and Wong, 1980) using kit from Spectrum Diagnostics, Egypt

Spot urine samples were used because it was shown that urinary protein/creatinine ratio (Chitalia et al., 2001), correlated with 24-h urinary excretion, eliminated variations due to changing rates of urine output, and provided a measurement independent of urine concentration. The ratio of the urinary concentration of amino acids to that of creatinine gives a more accurate measure of the re-absorptive efficiency of the tubule for amino acids during the period of collection, and provides a standard of comparison for amino acid data collected at different times.

5- The data was statistically analyzed using the “Statistical Package for Social Science (SPSS) version 18 Inc., Chicago, IL, U.S.A.”. Independent t-test, analysis of variance (ANOVA) were used to detect the statistical differences in the quantitative data between the three groups. Pearson’s bivariate correlation coefficient was also calculated. The differences were considered significant at a level of p < 0.05.

Results:

There were no significant differences between the exposed and the control groups as regards their ages. Also, there were no significant differences between the exposed groups (silicotics and non-silicotics) regarding the work duration.

Data from the present investigation (Fig1a,b) showed a high significant increase in the urinary excretion of all measured urinary parameters in both groups of silica-exposed workers compared with control subjects. Also, there is a high significant increase in the levels of measured urinary parameters of silicotics compared to non-silicotic exposed workers.

We compared the excretion of a number of physiologically linked amino acids according to known transport systems into four groups (Milne 1964).

1. Monoamino-monocarboxylic amino acids: Alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, histidine, methionine.

2. Dibasic amino acids: Lysine, arginine, and cystine.

3. Dicarboxylic amino acids: Glutamic and aspartic acids.

4. The imino-acid and glycine group: Proline and glycine.

The excreted seventeen amino acids investigated were: alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, histidine, methionine, lysine, arginine, and cystine, glutamic and aspartic acids, proline and glycine. Only four amino acids: cystine, methionine, isoleucine and arginine, could not be detected as their levels were very low to be measured. The mean of total amino acid excretion, adjusted for creatinine, was higher among both groups of the silica-exposed workers (1846.54±163.52µg/g (for silicotics); 964.81 ±78.75 (for non-silicotics) than the referents (314.62 ± 36.07) for the 13 compared amino acids (Fig 2). Also, the levels of individual amino acids excretion were significantly higher among both groups of the silica-exposed workers than the referents. In the silicotic group, the differences in the levels of excretion were statistically significantly higher for all the amino acids compared to the non-silicotic group except for valine and leucine. The comparison data for these amino acids are listed in Fig 3 (a,b).

In the silica- exposed group (n= 47) all groups of measured urinary amino-acids were significantly correlated with each other. Moreover, in each separate group most of the included amino acids were significantly correlated with each other (Table I).

There was significant correlation between urinary excretion of total amino-acids on one side and each of the following parameters U-Malb (r=0.635, p<0.01); U α1-M (r=0.411, p<0.01); U-NAG (r=0.503, p<0.01); U-zinc (r=0.384, p<0.01); U-silica (r=0.330, p<0.05). However, there was no significant correlation between urinary excretion of total amino-acids and either U- copper or duration of exposure.
Discussion:

An excessive industrial exposure to silica and an elevated silica content in renal tissue was found to have distinctive nephropathy, characterized pathologically by changes in the glomeruli and proximal tubules, and manifested clinically by albuminuria and hypertension (Saldanha et al., 1975). As the precise mechanisms of silica toxicity, and the direct toxic effects or immunological injury, are not clear, mechanistic studies have suggested that fresh surface of silica is highly reactive with hydrogen, oxygen, and carbon, driving oxidant production increasing the ability to produce free radicals in vivo (Fubini and Wallace, 2000).

In previous studies, results of investigating glomerular function supported reports that, silica exposure resulted in glomerular-type proteinuria with significant elevation of urinary albumin in the silica-exposed workers (Ng et al., 1992; Boujemaa et al., 1994; Hotz et al., 1995; El-Safty et al., 2003, Ibrahim et al. 2011). Glomerular capillary epithelial cells are covered with a polyanionic sialoprotein coat which is thought to repel polyanionic proteins and prevent passage of serum proteins into urinary space (Chang et al., 1975).

Maintenance of the integrity of the sialoprotein is presumably a function of epithelial cell, and damage to this system could result in proteinuria. Thus, finding elevated U-Malb in the present study suggested that epithelial cell injury could result in alterations in the molecular integrity of the sialoprotein. This injury could result from elevated silica content of the kidney (Hauglustaine et al., 1980), confirmed by elevated urinary silica level in the exposed workers in the present study and as reported by some authors (Hotz et al., 1995; El-Safty et al., 2003 and Ibrahim et al., 2011). Ibrahim et al. (2011) suggested renal proximal tubular damage in the silica-exposed workers. The proximal tubular injury may result in an increased urinary excretion level of U-Malb /U.cr. This suggestion is supported by the significant correlation between U-Malb/U.cr and each of α1-M/U.cr and U.NAG/U.cr.

It was reported that, the majority of the tubular changes resulting from silica exposure were confined to proximal convoluted tubules, which showed considerable structural alteration with much desquamation of cells (Bolton et al., 1981) and because amino acids are fully filterable at the glomerulus and more than 95% of the filtered load is reabsorbed in the proximal convoluted tubules (Watts, 1990), it could be suggested that loss of amino acid-metal complexes in urine due to tubular re-absorption dysfunction and tubular damage, may contribute to the elevation of the urinary excretion of amino acids. This suggestion was confirmed by the presence of the significant correlation between the urinary excretion of total amino-acids on one side and each of the following parameters

U-Malb; U α1M; U-NAG; U-zinc; U-silica on the other side.

In the present work, no association was found between the measured urinary parameters and duration of exposure to silica. Similarly, Ibrahim et al. (2011), Mwangi et al. (2009), Rosenman et al. (2000) and Boujemaa et al. (1994) didn’t find the same association. This absence of a dose-response relationship contrasts with the findings of Ng et al. (1992) who found a relationship between the prolonged exposure to silica and renal abnormalities in the form of raised concentrations of albumin and α1M in the urine. These functional changes were not reversible after removal from exposure. Vuppuluri et al. (2012) found strong specific association between occupational silica exposure and renal insufficiency with a dose-response trend. Additionally, they suggest that exposure to silica may be associated with earlier stages of the kidney disease.

Under physiologic circumstances, only minimal amounts of amino acids are excreted. In most mammals, 99% of filtered amino acids are reabsorbed in the proximal tubule and fractional excretions of most amino acids are between 0.2% and 2.5% (Dantzler and Silbernagl 1988). The results of our study suggested that, aminoaciduria was due to renal cause. The excretion of nearly all the amino acids was grossly raised and the aminoaciduria was generalized. This suggestion was confirmed by the presence of the significant correlation between all different measured urinary amino-acids groups and the correlation between most of the included amino acids in each group separately in the silica-exposed group. However, certain amino acids showed the greatest increase in excretion.

An increased urinary amino acids excretion could arise either through disturbed intermediary metabolism with raised plasma levels or by impaired re-absorption in the renal tubular cells. Since we did not measure serum amino acids, one could argue that the aminoaciduria exhibited by the exposed workers might arise from increased intake or impaired catabolism rather than from decreased tubular re-absorption. However increased dietary intake is unlikely because the studied groups were culturally similar. Thus, future studies are warranted to examine the association between silica exposure and the level of kidney functions to overcome the possible limitation of our study resulting from the small sample size.
Fig. 1-a: Levels of the various investigated urinary parameters among the studied groups

Fig. 1-b: Level of urinary silica among the studied groups
Fig. 2: Total amino acids excretion among the three groups

Fig. 3-a: Excretion of some amino acids in the target groups
Fig. 3-b: Excretion of the other amino acids in the target groups

Table I: Correlation coefficient of the investigated amino acids in the silica-exposed workers

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Alanine</th>
<th>serine</th>
<th>threonine</th>
<th>valine</th>
<th>leucine</th>
<th>lysine</th>
<th>histidine</th>
<th>tyrosine</th>
<th>glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serine</td>
<td>0.338</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>threonine</td>
<td>0.223</td>
<td>0.399</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>valine</td>
<td>0.581</td>
<td>0.562</td>
<td>0.772</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>0.334</td>
<td>0.563</td>
<td>0.852</td>
<td>0.892</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.183</td>
<td>0.286</td>
<td>0.652</td>
<td>0.768</td>
<td>0.702</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tyrosine</td>
<td>0.094</td>
<td>0.904</td>
<td>0.715</td>
<td>0.657</td>
<td>0.783</td>
<td>0.909</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>histidine</td>
<td>0.249</td>
<td>0.908</td>
<td>0.808</td>
<td>0.781</td>
<td>0.882</td>
<td>0.631</td>
<td>0.721</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>lysine</td>
<td>0.294</td>
<td>0.571</td>
<td>0.812</td>
<td>0.774</td>
<td>0.852</td>
<td>0.525</td>
<td>0.671</td>
<td>0.840</td>
<td>1</td>
</tr>
<tr>
<td>glutamic</td>
<td>0.556</td>
<td>0.566</td>
<td>0.599</td>
<td>0.740</td>
<td>0.855</td>
<td>0.641</td>
<td>0.630</td>
<td>0.045</td>
<td>0.671</td>
</tr>
<tr>
<td>aspartic</td>
<td>0.592</td>
<td>0.025</td>
<td>0.783</td>
<td>0.854</td>
<td>0.864</td>
<td>0.582</td>
<td>0.849</td>
<td>0.768</td>
<td>0.787</td>
</tr>
<tr>
<td>proline</td>
<td>0.376</td>
<td>0.563</td>
<td>0.604</td>
<td>0.740</td>
<td>0.777</td>
<td>0.484</td>
<td>0.845</td>
<td>0.558</td>
<td>0.883</td>
</tr>
<tr>
<td>glycine</td>
<td>0.332</td>
<td>0.635</td>
<td>0.860</td>
<td>0.859</td>
<td>0.928</td>
<td>0.961</td>
<td>0.784</td>
<td>0.819</td>
<td>0.785</td>
</tr>
</tbody>
</table>

Linear correlation
*P<0.05
**P<0.01

Conclusion:

A “generalized” aminoaciduria may occur in silica-exposed workers. Profiling of amino acid availability is relevant to detoxification capacity. It was evident that the occurrence of amino aciduria would be an important investigation of the toxic effects of the silica exposure. These results provide confirmatory evidence of the relationship between occupational silica exposure and renal disorders.

References


