Further evidence of human silica nephrotoxicity in occupationally exposed workers

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Abstract

It has previously been shown that granite workers with heavy exposure to silica had glomerular and proximal tubular dysfunction evidenced by increased urinary excretions of albumin, α -1-microglobulin (AMG), and β -N-acetyl-glucosaminidase (NAG). The investigation was replicated in another group of granite workers to further elucidate the exposure effect relation. The urinary excretion of albumin, α -1-microglobulin (AMG), β -2-microglobulin (BMG), and β -N-acetyl-glucosaminidase (NAG) was determined in two groups of granite workers with low and high exposure to silica. Low molecular weight proteinuria and enzymuria were significantly correlated with duration of exposure in the high but not the low exposure group. These increases were most pronounced in those with 10 or more years of heavy exposure, and in those with radiological evidence of pulmonary fibrosis, particularly those with rounded small opacities denoting classical silicosis. These results provide further evidence that prolonged and heavy exposure to silica is associated with nephrotoxic effects in granite workers.

(British Journal of Industrial Medicine 1993;50:907-912).

The evidence that silica is nephrotoxic in occupationally exposed persons is limited. Anecdotal reports and uncontrolled studies of clinical and pathological case series suggest that severe pulmonary silicosis, especially acute silicosis, is associated with glomerulotubular disease.¹⁻⁸ We have also reported preliminary findings from a recent cross

sectional study of an increased urinary excretion of albumin, α -1-microglobulin (AMG), and β -N-acetyl-glucosaminidase (NAG) in granite workers with high exposure to silica compared with healthy unexposed subjects.⁹ Some of these alterations in renal function, particularly for albumin and NAG, were apparently greater and persistent in silicotic subjects who were assumed to have had greater exposure to dust and who had not been exposed to silica for many years. We have replicated the study in another larger group of granite workers, with the further aim of elucidating the exposure-effect relation between the urinary alterations on the one hand and the intensity and duration of exposure to silica on the other.

Materials and methods

The study subjects were selected from among a total of 159 workers in three granite quarries (not covered in the previous study), who held jobs with differing intensities of exposure to dust. A high dust exposure group of workers included those who were employed regularly in rock drilling and rock crushing jobs for at least a year; whereas a low exposure group included the rest of the workers who were mostly employed in equipment and vehicle maintenance and servicing work in the workshops, as loader operators and dumper truck drivers, and other administrative workers (clerks, office assistants, storemen etc). The exclusion criteria were a known history of glomerulonephritis, urinary calculi, or other renal diseases or diabetes, hypertension, or regular recent ingestion of analgesics. A total of 21 subjects was excluded in this way, and from among the rest of the workers who provided suitable specimens of urine (see later), a random one in two sample of 67 subjects was eventually studied.

Samples of urine were collected from the subjects in the morning from 10.00 am to 12.00 noon. The pH was determined immediately after collection and all samples for BMG estimations were neutralised immediately with a standard buffered solution. The samples were stored frozen and analysed within one month of collection. Urinary

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Table 1 Basic characteristics and urinary indices by exposure group

	Low exposure	High exposure	p Value
No	31	36	
Race:			
Chinese (%)	26 (83.9)	34 (94.4)	
Malays (%)	4 (12.9)	2 (5.6)	
Indians (%)	1 (3.2)	0 (0.0)	NS
Age (y)	32.8 (6.2)	36.6 (11.1)	NS
Years employed	8.6 (5.1)	12.5 (10.2)	0.049
Current smokers:			
No (%)	10 (32.3)	17 (47.2)	
Cigarette-years	195 (172)	309 (324)	NS
Ex-smokers:			
No (%)	7 (22.6)	4 (11.1)	
Cigarette-years	133 (162)	287 (151)	NS
Urinary creatinine (g/l)	1.58 (0.82)	1.45 (0.65)	NS
Urinary AMG (mg/g creatinine)	3.10 (2.00)	5.46 (3.05)	0.0003
Urinary BMG (mg/g creatinine GM (GSD))	61.6 (1.86)	89.1 (2.19)	0.038
(Range)	(5.3-376.4)	(10.9-987.8)	
Urinary albumin (mg/g creatinine)	5.83 (3.23)	6.46 (3.29)	NS
Urinary NAG (U/g creatinine)	1.56 (0.81)	2.26 (1.72)	0.034

Values are mean (SD) unless stated otherwise; GM, geometric mean; GSD, geometric standard deviation.

albumin was measured with an immunoprecipitation assay kit (Orion Diagnostica, Finland), AMG and BMG were analysed with enzyme-immunoassay kits (Fujirebio Inc, Japan), and NAG was analysed with a colorimetric assay (Boehringer-Mannheim, Germany). Urinary concentrations of these substances were corrected for urinary creatinine. Samples with pH below 5.5 and urinary creatinine below 0.3 g/l were excluded.

At the same time large sized chest radiographs of all the workers were taken and the films were read by three experienced physician readers, independently and in random order, without knowledge of the workers' personal and exposure particulars. The results of these radiographic readings have been reported in another paper,¹⁰ and the majority readings of the three readers were used to classify each of the 67 subjects in this study as being pneumoconiotic or non-pneumoconiotic. Radiological pneumoconiosis was defined as rounded or irregular small opacities equal to or greater than 1/0.

Logarithmic transformation was used to normalise the distribution of values for BMG, and Student's *t* test and χ^2 tests of significance were used as appropriate. A *p* value <0.05 was considered significant.

Results

The men in the high exposure group (drilling and crusher workers) did not differ significantly from the men in the low exposure group (maintenance, transport, and administrative workers) in respect of age, race, and smoking habits (table 1). There was no significant relation of these variables with any of the urinary indices of renal function. The urinary creatinine concentrations in both groups were similar, but the concentrations of AMG, BMG, and NAG, corrected for creatinine, were significantly higher in the high exposure group. The concentration of albumin, was, however, not significantly increased in the high exposure group.

Table 2 Correlation coefficients (*r*) of exposure and urinary indices by exposure group

Indices		Low exposure		High exposure	
		<i>r</i>	<i>p</i> Value	<i>r</i>	<i>p</i> Value
Age	Job-years	0.58	0.001	0.71	0.0001
	AMG	-0.01	NS	0.27	NS
	Log BMG	-0.03	NS	0.15	NS
	NAG	0.24	NS	0.52	0.0011
	ALB	0.19	NS	-0.01	NS
Job-years	AMG	0.01	NS	0.33	0.047
	Log U BMG	-0.10	NS	0.40	0.015
	NAG	0.23	NS	0.55	0.0005
	ALB	0.24	NS	-0.03	NS
	Log BMG	0.36	0.048	0.64	0.0001
AMG	ALB	-0.21	NS	0.05	NS
	NAG	0.24	NS	0.50	0.019
	ALB	0.24	NS	0.18	NS
Log BMG	NAG	0.39	0.03	0.46	0.005
	ALB	0.21	NS	-0.13	NS

Values of AMG, BMG, and NAG measured in urine.

Table 3 Urinary indices by exposure groups and duration of exposure

	Low exposure	High exposure	
		<10 yr	≥ 10 yr
No	31	17	19
Urinary AMG (mg/g creatinine)	3.10 (2.00)	4.99 (2.19)	5.88 (3.66)*
Urinary BMG (mg/g creatinine; GM (GSD))	61.6 (1.86)	72.4 (2.14)	107.1 (2.24)*
(Range)	(5.3-376.4)	(10.9-258)	(30.2-987.8)
Urinary Alb (mg/g creatinine)	5.83 (3.23)	6.19 (2.94)	6.69 (3.64)
Urinary NAG (U/g creatinine)	1.56 (0.81)	1.71 (0.86)	2.75 (2.14)*

* $p < 0.05$ by Bonferroni (Dunn) t tests. Abbreviations as for table 1.

The relation of these urinary indices with duration of exposure in years was examined in the two groups (table 2). As expected, years of exposure was highly and significantly correlated with age in both groups. It was interesting to note, however, that in the low exposure group, neither age nor years of exposure was significantly correlated with any of these urinary indices, whereas in the high exposure group, exposure-years was significantly correlated with urinary AMG, BMG, and NAG. It was also interesting to note that urinary AMG, BMG, and NAG concentrations were all significantly correlated with one another.

The magnitude of the differences in urinary indices by both intensity and duration of exposure was further examined (table 3). Subjects who were exposed to high dust concentrations for 10 or more years clearly showed the most significant increase in urinary concentrations of AMG, BMG, and NAG. Subjects with less than 10 years of heavy exposure generally also had higher concentrations of these proteins and enzyme than workers in the low exposure group, although the differences were not statistically significant.

Further analyses involved the examination of the relation between exposure and radiological pneumoconiosis on the one hand and concentrations of urinary proteins and enzymes on the other. In this analysis, the subjects were further divided into three groups: a low exposure group with no radiological pneumoconiosis (mean (SD) age 32.2 (5.2), exposure duration 8.3 (4.7) years); a high exposure group with no radiological pneumoconiosis (mean (SD) age 33.4 (9.1), exposure duration 9.6 (8.2) years); and a group with radiological pneumoconiosis (mean (SD) age 44.2 (11.7), exposure duration 18.6 (11.2) years) (figure). Those with pneumoconiosis included five with irregular small opacities, denoting "mixed dust fibrosis" (mean (SD) age 38.2 (13.2), exposure duration 13.2 (6.4) years), and seven with rounded small opacities, denoting "classical silicosis" (mean (SD) age 46.6 (9.3), exposure duration 22.4 (12.8) years), all in radiological profusion categories 1/0 to 1/2. The results showed the highest increases in urinary concentrations of AMG ($p < 0.01$), BMG ($P < 0.01$), and

NAG ($p < 0.01$) in the group with radiological evidence of pulmonary fibrosis. It is interesting to note that low molecular weight proteinuria and enzymuria were more pronounced for those with classical silicosis compared with those with mixed dust fibrosis.

Discussion

The urinary excretion of high and low molecular weight proteins and enzymes are recognised to be useful markers of early glomerular and tubular dysfunction, and are highly sensitive tools for screening of industrial nephrotoxicity.¹¹ The measurement of urinary BMG is a widely used test of early renal tubular deficiency, but its drawbacks are well known. It is easily degradable at urinary pH below 5.5, and this degradation starts in the bladder. For these reasons, special measures were taken to ensure optimal pH conditions of urine specimens before analyses. It was hence reassuring to note that the urinary BMG values were significantly correlated with that of another low molecular weight protein, AMG. α -1-Microglobulin is a new indicator protein that has proved useful for the early detection of renal tubular dysfunction caused by environmental exposure to cadmium,¹²⁻¹⁴ and which also possesses the distinct advantage of being stable at urinary pH between 4 and 10. Both low molecular weight proteinuria were correlated with urinary excretion of NAG, and these altogether provide strong assurance of the self-consistency and validity of these indices of renal tubular function.

The results of the present study confirmed previous findings of a nephrotoxic effect associated with long term occupational exposure in granite workers. Also, the additional information in this study provided further elucidation of the exposure effect relation. Clear evidence of renal tubular dysfunction was found in workers with 10 or more years of heavy exposure. These functional changes hence arise from prolonged and heavy exposure to dust, and as suggested by the results of our previous study of silicotic patients who had ceased exposure for many years may reflect irreversible

Urinary indices in low and high exposure and pneumoconiosis groups. Group 1 ($n = 28$): low exposure, no radiological opacities. Group 2 ($n = 27$): high exposure, no radiological opacities. Group 3 ($n = 12$): radiological opacities with profusion $\geq 1/0$. Solid bar denotes arithmetic mean except for BMG (geometric mean). Dashed bar denotes mean for subjects with classical silicosis (closed triangles).

damage. The urinary results in those with low dust exposure seemed to be normal, as they were essentially similar to the levels in the group of healthy subjects with no dust exposure in the previous study.

In our previous study, urinary excretion of albumin was significantly increased in subjects with heavy exposure to silica. In the present study, however, we were not able to replicate these results. Nevertheless, the possibility of glomerular disturbance cannot be excluded. Among other things, this might be due to differences in the characteristics of the subjects who were selected in the two studies. The highly exposed group of drilling and crushing workers in the present study were exposed for an average of 12 years, whereas the mean duration of exposure for a similar group of workers in the previous study was 16 years. The previous study also included more cases with greater severity of silicosis. It is possible that glomerular dysfunction might be evident subsequent to renal tubular disorder.

The possibility of unrecognised renal diseases of known pathology may be considered as an alternative explanation for the results. Urinary calculi were thought to be common among these granite workers because they performed strenuous work under an extremely hot outdoor environment, and it is possible that symptom free urinary calculi might explain these results. A systematic examination of the prevalence of known passage of urinary calculi in a larger group of these workers, however, did not suggest that those who were more heavily exposed to silica had higher risks than those less exposed.¹⁵

It is also possible that the urinary alterations in highly exposed workers might be explained by other potential nephrotoxic agents apart from silica in the granite quarrying work environment. Such exposures possibly include diesel and other petroleum based oils and lubricants, commonly used on machines and vehicles. In the low exposure group, loader operators and truck drivers, as well as some machine mechanics were in fact likely to be equally or more heavily exposed to these industrial substances than rock drillers or crusher workers. A thorough evaluation of environmental exposures in the granite industry that pose nephrotoxic hazards is made difficult by the present limited knowledge of the potential human nephrotoxicity of many industrial substances in use today. On the other hand, it is interesting to note the presence of low molecular weight proteinuria and enzymuria in subjects with radiological evidence of pulmonary fibrosis, which was most pronounced in those with classical silicosis, by contrast with subjects with mixed dust fibrosis. This finding favours an association with exposure to quarry dust, and as a high fractional content of free silica is typically associ-

ated with classical silicosis, points at the same time towards a specificity for silica.

Although there are epidemiological studies of mortality in cohorts of workers exposed to silica, only a few reports provided data on deaths due to renal diseases, probably because of the few cases found. In our own cohort of 2000 silicotic subjects, we have also found only two deaths (expected 4.07) from genitourinary causes.¹⁶ A proportionate mortality study of Vermont granite workers¹⁷ found no statistically significant excesses of deaths overall from genitourinary causes (15 observed, 13.3 expected), but an excess of genitourinary disease (five observed, 1.8 expected) was noted in a subgroup of other quarry workers. A cohort study of Finnish granite workers¹⁸ found four cases of diseases of the urinary tract (1.8 expected), three of which were pyelonephritis (expected 0.6), and one chronic nephritis. Five other cases of renal disease were reported as contributing causes of death.

We conclude that prolonged heavy exposure to silica in granite workers is nephrotoxic, but, the clinical significance of these functional changes is still unclear. The risks of clinically or pathologically evident renal disease in persons exposed to silica should be actively investigated in future studies.

We thank Miss Ng Yuen Ling for her assistance with the laboratory analysis in this study.

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Accepted 14 December 1992