Liver functions in silica-exposed workers in Egypt: possible role of matrix remodeling and immunological factors

Nermin Zawilla¹, Fatma Taha², Yasser Ibrahim³

¹Occupational and Environmental Medicine, Faculty of Medicine, Cairo University, Egypt, ²Medical Biochemistry, Faculty of Medicine, Cairo University, Egypt, ³Air Pollution Department, National Research Center, Cairo, Egypt

Background: Brick manufacturing constitutes an important industrial sector in Egypt with considerable exposure to silica.

Objectives: We aimed for evaluating hepatic functions in silica-exposed workers in the clay brick industry, and the possible role of matrix remodeling and immunological factors.

Methods: A case-control study, 87 workers as exposed and 45 as control subjects. Questionnaire, clinical examination, and laboratory investigations: liver functions, matrix metalloproteinase-9, immunoglobulins G and E, and anti-liver kidney microsomal antibody.

Results: In the exposed workers, mean levels of liver functions, matrix metalloproteinase-9 (MMP-9), and IgG and IgE were significantly higher. In the silicotic subgroup the mean level of GGT was almost twice the level in the non-silicotic subjects. Logistic regression showed that abnormal GGT and ALT were associated with production workers.

Conclusion: Workers in the clay brick industry showed evidence of liver disease that could be related to matrix remodeling.

Keywords: Brick workers, Silica, Liver functions, Matrix metalloproteinase-9, Immunoglobulins

Introduction

Silica or silicon dioxide is an abundant mineral found in rocks, sand, and soil. Silica primarily exists in its crystalline state as quartz, which is structurally and chemically different from amorphous silica (e.g. diatomaceous earth), silicates (e.g. talc or asbestos), and silicone. Ubiquitous in the environment, silica is part of the small particulate fraction of air pollution and may comprise a significant fraction of environmental dust levels in some geographic regions.¹

Occupational exposures to silica are diverse and include manufacturing and construction processes that use silica as a tool or a raw material, as well as the mining and processing of silica-containing rocks. Millions of workers are exposed to silica worldwide with increased mortality and morbidity, making it a high-priority public health concern in both developing and developed nations.²

In Egypt, brick manufacturing is an important industry, employing approximately 1 million temporary or permanent workers.³ The typical components of clay

brick are silica (50–60% by weight), alumina (20–30%), and lime (2–5%). During the industrial process considerable amounts of silica dust and smoke are generated.⁴

There is a strong epidemiological evidence to support a causal association between occupational crystalline silica exposure and several respiratory diseases including: silicosis, lung cancer, pulmonary tuberculosis, chronic obstructive pulmonary diseases, autoimmune (systemic sclerosis, systemic lupus erythematosus, and rheumatoid arthritis), and renal diseases.^{1,2} Silicosis is characterized by respiratory damage that ranges from reversible functional changes to irreversible damage of the lungs.⁵ The most important factors in the development of silicosis are the dose, percentage, and crystalline nature of silica contained in the inhaled dust.⁶

The transport of silica particles after inhalation or ingestion can result in their widespread systemic dissemination. Systemic effects of silica include hepatic or hepatosplenic silicosis and granulomas, hepatic porphyria, cutaneous silica granulomas, and dental abrasions.⁶ However, the observation of extrapulmonary effects comes mainly from occupational settings in which exposures have been heavy enough to result in

Correspondence to: Dr. Nermin Hamdy Zawilla, Occupational and Environmental Medicine, Faculty of Medicine, Cairo University, El Saray street, Manial, Cairo, Egypt. Email: nerhamdy71@hotmail.com

silicosis.⁷ The silicon content of organs has sometimes been elevated in patients dying of silicosis, with silicotic nodules found in the liver, spleen, and bone marrow.⁸

Several theories and mechanisms have been postulated in the pathogenesis of silica-related diseases and immunological mechanisms that alter the humoral immune functions and hypergamma-globulinemia in the presence of autoantibodies and circulating immune complexes have been confirmed.^{9,10} There is *in-vivo* evidence for both enhancement and depression of immune responses after exposure to silica.¹¹

Extracellular proteinases regulate development and physiologic events, including branching morphogenesis, angiogenesis, wound healing, and extracellular matrix (ECM) degradation. The matrix metalloproteinases (MMPs) are families of ECM degrading enzymes that share common functional domains and activation mechanisms.¹² In considering the domain structure and/or substrate affinity, four major subgroups have been defined: collagenases, stromelysins, gelatinases, and membrane-type metalloproteinases. Gelatinases (MMP-2 and MMP-9) have a substrate affinity for basement membrane type IV collagen, denatured collagens (gelatin), and elastin.¹³

Normal ECM remodeling depends on a balanced synthesis/degradation pattern. Extracellular matrix degradation is a complex multistep process that involves MMPs. Matrix metalloproteinase-9 has been implicated in scarring and fibrosis following myocardial, liver, and kidney injuries. Furthermore, previous research has found that MMP-9 gene deletion results in reduced interstitial fibrotic lesions in mice, and inhibits allergen or injury-induced lung and liver fibrosis.^{14–17}

Occupational and environmental exposures to hepatotoxins are a risk to the liver. The possible additional effect of silica exposure on the liver was the target of our study. The primary aim of this study was to evaluate the hepatic functions among clay brick workers occupationally exposed to silica. The secondary aim was to investigate the possible role of matrix remodeling (through measurement of matrix metalloproteinase-9) and immunological mechanisms (through measurement of immunoglobulins G and E, and anti-liver kidney microsomal antibody) in the pathogenesis of this effect. This is the first study of its kind to be carried out among clay brick workers in Egypt. It adds to existing literature by further exploring the possible systemic effects of silica exposure.

Methods

Subjects

A total of 132 male workers participated in this case-control study: 87 workers occupationally exposed to silica and 45 unexposed workers as their referent

controls (Fig. 1). Exposed workers were recruited from a clay brick factory comprised of a mining and production sector in the Helwan area in Southern Cairo, Egypt. Clay mining (or winning) is dependent on the depth, thickness, hardness, and physical geology of the clay beds and extraction is performed using heavy machinery to stockpile large amounts of clay. The production sector is responsible for the crushing, milling, mixing, casting, and firing of bricks. In total, 140 workers were employed in the factory. All eligible employees were invited to participate in the study. Eligibility criteria for exposed workers included being employed in the clay brick factory during the preceding 5 years and having no other previous work history. The control subjects were security personnel and administrative workers in a small insurance company, matched to the exposed group by age, body mass index (BMI), and smoking status. The controls did not reside in the same industrial area as the exposed workers and were never occupationally exposed to silica dust or hepatotoxins.

Exclusion criteria for both the exposed and control groups were: any history of alcohol consumption, BMI > 30, use of drugs with possible hepatotoxic effect, diabetes, uncontrolled hypertension, current or previous viral hepatitis, schistosomiasis, or an autoimmune disease.

After eligibility screening, 53 of the occupationally exposed workers were excluded from the study (Fig. 1).

Ethical consideration

All the included subjects were treated according to the Helsinki Declaration of Biomedical Ethics and provided informed consent before study participation.¹⁸

Methods

The study involved two main components:

I-Biological Study

A full medical history and clinical examination were completed for all participants. We designed a questionnaire to collect demographic and medical data, smoking history, and occupational history (type and duration of work). Body mass index was calculated for each subject.

A chest X-ray was performed on workers occupationally exposed to silica dust and the radiographs were provisionally interpreted. Workers showing radiological evidence of pneumoconiosis were transferred to the Occupational Medicine Department at the National Health Insurance Authority, where a second confirmatory X-ray and pulmonary function tests were performed. Two independent radiologists interpreted chest X-rays and participants were provided with an occupational disease and disability assignment when applicable.

Criteria for silicosis diagnosis and compensation assignment were based on radiographic findings consistent with pneumoconiosis (according to ILO

Figure 1 Flow chart of subject selection for exposed and control groups.

guidelines),¹⁹ a verified history of occupational exposure to silica dust, and a level of general working disability reflecting a decline in respiratory functions as measured by traditional tests of pulmonary function: spirometry and lung volume measurements. Spirometry included the forced vital capacity (FVC), forced expiratory volume in 1 second (FEV-1), and

peak expiratory flow rate (PEFR). Lung volume measurements included maximum voluntary ventilation (MVV), total lung capacity (TLC), vital capacity (VC), and residual volume (RV).

In total, 35 of the 45 referred cases were granted a disability rating by the Higher Committee for Occupational Disability Evaluation.

All study subjects were screened for viral hepatitis B and C and the following biochemical tests were performed among participants with negative results:

- (i) immunoglobulins G and E,
- (ii) liver kidney microsomal antibody
- (iv) matrix metalloproteinase-9,
- (v) liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, and albumin.

Sample collection and analysis

Venipuncture was used to collect blood into plain tubes. Samples were allowed to clot for 2 hours at room temperature and then centrifuged for 20 minutes at 1000 revolutions per minute (rpm). Serum was stored at -80°C until assay.

Biochemical measurements assessed hepatic functions associated with necrosis, cholestasis, or altered hepatic clearance. Tests of liver functions associated with necrosis included the serum hepatic transaminases ALT and AST activity in serum obtained by standard clinical laboratory methods.²⁰ Tests of hepatic cholestasis included ALP and gamma glutamyl transferase (GGT).^{20,21} Hepatic clearance was assessed by measurements of total bilirubin concentration using standard diazo reaction method.²² Synthetic function of the liver was assessed by the measurement of albumin.²³ Automated analyzer Hitachi 917 was used in the analysis and chemicals were provided by Bio Med EGY-Chem (Cairo, Egypt).

The 95th percentile values for hepatic functions in the laboratory reference population were: ALT > 29 U/l; AST > 25 U/l; GGT > 50 U/l; ALP > 220 U/l; total bilirubin 1.1 mg/dl; and albumin > 5 g/dl.

Serum concentration of MMP-9 was measured using MMP-9 Human Elisa Assay (Boster Biological Technology Ltd., Fremont, CA, USA).²⁴

Serum IgG was measured using IgG Human Elisa Assay (USCN Life Science Inc. Wuhan China).²⁵ Serum IgE was assessed using IgE Human Elisa Assay (BioCheck Inc., Foster City, CA, USA).²⁶

Liver kidney microsomal antibody type 1 was measured using the indirect immune fluorescence method (IFL) (Astra diagnostici, Milano, Italy).²⁷

II-Environmental Study²⁸

Sampling locations and durations

Air samples were collected in the breathing zones of workers and as near as possible to the working stations in the clay brick factory. The sampling pumps were set approximately 1.5 m above ground level, with two sites selected in each of the production, mining, and control areas, reflecting the main sites for possible dust exposure. Control air samples were collected from two different office wards at the

insurance company where controls were recruited. Air sampling pumps operated for 8 hours, with sampling taking place biweekly (Monday and Thursday) for four consecutive weeks. A total of 48 samples were collected from the selected sites.

To measure respirable dust, personal sampler devices were attached to the belts and collars of exposed workers. Sampling pumps were calibrated before and after sampling using a Gilibrator bubble flow meter to verify that the air-sampling rate had remained constant. The personal samples were collected on the same days as the area samples and were performed biweekly for two consecutive weeks. A total of 48 personal samples were collected and analyzed.

In addition to measurements of the air quality, we performed walkthrough inspections in the production and mining areas of the brick factory. Workers were observed throughout the shift and detailed information on tasks performed, task duration, and processes (manual versus mechanical) were recorded. Twelve workers (three workers from each of the two shifts in the production and mining areas) responsible for tasks representative of jobs identified as at-risk for silica exposure were selected for observation.

Sampling techniques

Total suspended particulate matter was measured at two mining, two production, and two control sites. To determine the quantity of particulate matter, membrane filters were weighed in the laboratory, mounted on a filter holder, and transported to the selected sites. Air was aspirated by a calibrated vacuum pump with a rate of 14 l/minute, and the dry gas meter readings were recorded before and after sampling period. The membrane filters were weighed after the sampling time and the difference in weight before and after sampling was recorded as the weight of suspended particulate matter (expressed in milligrams per cubic meter). Air volume was calculated from the dry gas meter readings.

The respirable dust sampling technique was used to measure the worker's exposure to free silica (crystalline). A portable battery operated pump equipped with a pulsation dampener was used. The pump drew 1.7 l of air/minute for at least 8 hours. Dust was collected with a size appropriate personal sampler positioned in the person's breathing zone. Personal samples were collected with a two-stage size selective sampling device. Air penetrates the precollector which is a 10 mm nylon cyclone. The precollector is connected to a 37 mm cassette with a 37 mm low ashing polyvinyl chloride filter (0.5 μm pore size) as the collecting medium.

After sampling, filters were capped, sealed, and stored in plastic bags until analyses. For quality

Table 1 Total dust, respirable dust, and free silica in the production and mining areas

	Production area	Mining area
Total dust (mg/m ³)	4.26 ± 1.54	9.97 ± 1.87*
Respirable dust (µg/m ³)	844.0 ± 536.0	1319 ± 562.0
Free crystalline silica (%)	17.5	17.1
Free crystalline silica concentration (µg/m ³)	140.4 ± 57.01	165.8 ± 79.8

*Highly significant difference $P < 0.001$.

control, one sample blank was taken for each 10 samples. Sampling pumps were calibrated before and after each measurement. The filters were equilibrated in an environmentally controlled weighing area for at least 3 hours and weighed before and after sampling on a microbalance. For each sample, the respirable dust concentration (in micrograms per cubic meter) was calculated using the difference in filter weight after adjustment for field blanks and sampling volume.

The free silica content of the collected dust was determined by X-ray diffraction, using a Philips X-ray diffraction equipment model PW/1710 with monochromator, cu radiation ($\lambda = 1.542 \text{ \AA}$) at 40 kV, 30 mA and scanning speed of $0.02^\circ/\text{second}$.

Statistical analysis

Data were analyzed using SPSS 15.0 for Windows (SPSS Inc, Chicago, IL, USA, 2006). Demographic data from the exposed and control groups were compared using two-tailed Student's *t*-test and chi-square tests as appropriate. Analysis of variance (ANOVA) with *post-hoc* test (Bonferroni) was used for multiple comparisons of the quantitative data between the two groups. Correlations were used to test for linear relations between quantitative variables using Pearson correlation analysis. Generalized linear model multivariate analysis tested for significant predictors of liver affection in silica-exposed workers. A binary logistic regression model, using worker's job as a miner or production worker as the dependent variable and abnormal liver functions and MMP-9 as the independent variables, was performed. The relative risk was represented by an odds ratio (OR) and 95% confidence intervals (CI). A *P*-value of less than 0.05 was considered statistically significant.

Results

Results from the environmental assessment study indicated that the mean levels of total suspended dust, respirable dust, and free crystalline silica were higher in mining area than in production area (Table 1).

Demographic data from the study population are presented in Table 2. The exposed and control workers were matched in age, BMI, and smoking history. The mean duration of employment for the exposed workers was 24.49 ± 7.45 years (range 5–35 years). On average, they worked for 12-hour shifts, 5 days a week. (We did not observe workers regularly using protective equipment OR workers did not report regularly using protective equipment).

In the sample, 51.7% of all exposed workers had abnormal radiological findings and were referred appropriately. Of these cases, 41.3% were diagnosed with silicosis.

One way multivariate analysis of variance (MANOVA) revealed a statistically significant multivariate main effect for exposure to silica (independent variable) [$F(9, 122) = 36.182, P < 0.001$; Wilk's $\lambda = 0.273$, partial $\eta^2 = 0.727$]. Power to detect this effect was 1.00, thus the hypothesis of silica effect was confirmed. Given the significance of the overall test, the univariate main effects were examined (tests of between-subjects effects). Significant univariate main effect for silica exposure was obtained for the dependent variables: MMP-9, IgG, IgE, ALT, AST, and GGT. MMP-9 ($F(1, 130) = 260.642; P < 0.001$; partial $\eta^2 = 0.667$), IgG ($F(1, 130) = 46.334; P < 0.001$; partial $\eta^2 = 0.263$), IgE ($F(1, 130) = 35.725; P < 0.001$; partial $\eta^2 = 0.216$), ALT ($F(1, 130) = 5.031; P = 0.02$; partial $\eta^2 = 0.037$), AST ($F(1, 130) = 30.412; P < 0.001$; partial $\eta^2 = 0.190$), GGT

Table 2 Demographic characteristics of the studied population

	Exposed group (87)	Control group (45)	<i>P</i>
Age (years) (mean ± SD)	47.36 ± 7.31	47.49 ± 6.72	*0.92
Height (cm) (mean ± SD)	174.01 ± 5.00	172.16 ± 5.31	*0.05
Weight (kg) (mean ± SD)	80.47 ± 3.10	80.44 ± 3.12	*0.96
*BMI (kg/m ²) (mean ± SD)	26.63 ± 1.72	27.22 ± 2.02	*0.08
Duration of employment (years) (mean ± SD)	24.49 ± 7.45	-	
Smoking (yes %)	17 (19.5%)	15 (33.3%)	**0.08
Miners (%)	27 (31%)	-	
Production workers (%)	60 (69%)	-	

*BMI: Body mass index.

**Chi square test

†*t*-test

Table 3 Liver functions, matrix metalloproteinase-9 (MMP-9), and immunoglobulins in exposed and control workers

	Exposed group (87) mean $\pm$ SD	Control group (45) mean $\pm$ SD	P
Albumin(g/dl)	4.15 $\pm$ 0.29	4.23 $\pm$ 0.37	0.12
ALT (U/l)	21.11 $\pm$ 12.49	16.77 $\pm$ 4.71	0.02
AST (U/l)	31.47 $\pm$ 18.53	16.05 $\pm$ 3.68	<0.001
ALP (U/l)	188.62 $\pm$ 54.57	169.24 $\pm$ 33.36	0.03
GGT (U/l)	95.97 $\pm$ 76.6	21.49 $\pm$ 8.22	<0.001
Bilirubin (mg/dl)	0.76 $\pm$ 0.22	0.734 $\pm$ 0.14	0.41
MMP-9 (ng/ml)	139.8 $\pm$ 36.44	49.76 $\pm$ 11.46	<0.001
IgG mg/dl	1395.52 $\pm$ 398.5	957.8 $\pm$ 227.9	<0.001
IgE IU/ml	237.13 $\pm$ 273.18	69.29 $\pm$ 22.326	<0.001

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, GGT: gamma glutamyl transpeptidase, MMP-9: matrix metalloproteinase-9, IgG: immunoglobulin G, and IgE: immunoglobulin E.

(F (1, 130)=42.066; $P<0.001$; partial $\eta^2=0.244$). Power to detect these effects was 1.000.

In the exposed workers, the mean level of liver function tests (except albumin and bilirubin), MMP-9, and immunoglobulins (G and E) was significantly higher in the exposed group compared to the control group (Table 3). The titer for anti-liver kidney microsomal antibody was negative (at a dilution of 1/20) in all subjects. Abnormal liver functions in the exposed workers were as follows: abnormal ALT (17/87, 19.5%), abnormal AST (50/87, 57.5%), abnormal ALP (17/87, 19.5%), and abnormal GGT (51/87, 58.6%) (data not shown).

Based on the presence of silicosis, the exposed workers were classified into silicotic group 1 ($n=36$, 41.4%) and non-silicotic group 2 ($n=51$, 58.6%), shown in Table 4. The exposed workers with silicosis were significantly older (51.03 ± 4.84 and 44.76 ± 7.68 years respectively, $P<0.001$) and had a longer duration of work compared to exposed workers with no silicosis ($P<0.001$). There was no significant difference in the BMI between the two groups. The mean disability rating in the silicotic group was 23.83 ± 8.02 (range 5–35%).

One way ANOVA was used to compare means between the exposed subgroups (Group 1 with

silicosis and Group 2 without silicosis), and the control group (Group 3). A statistically significant difference was found between groups regarding liver functions (with the exception of albumin and ALP), MMP-9, and immunoglobulins.

A *post-hoc* test (Bonferroni test) for the ANOVA showed that the mean levels of AST and GGT were significantly higher in the exposed subgroups versus the control group (while mean level of ALT was significantly higher in the non-silicotic group only). In the silicotic subgroup (Group 1), the mean level of GGT was almost twice the level in the non-silicotic subjects ($P<0.001$), and the bilirubin was also significantly higher ($P<0.05$). Matrix metalloproteinase-9 and immunoglobulins G and E were significantly higher in each of the exposed subgroups compared to the referent control ($P<0.001$).

Pearson correlation between different variables in the exposed group (Table 5A and B) revealed a significant positive correlation between ALT and smoking index ($P=0.002$) and between AST and the duration of employment ($P=0.034$). Albumin was significantly negatively correlated with duration of employment ($P=0.04$). Matrix metalloproteinase-9 was significantly positively correlated with duration of employment and age of the exposed workers ($P<0.001$).

Table 4 Demographic data, liver functions, matrix metalloproteinase-9 (MMP-9), and immunoglobulins in the silicotic, non-silicotic, and control groups

	Silicotic (36) group 1	Non-silicotic (51) group 2	Control (45) group 3	F df (2,128)
Age (years)	51.03 $\pm$ 4.84	44.76 $\pm$ 7.68 ^{b**}	47.49 $\pm$ 6.724	9.22
Duration of employment (years)	27.61 $\pm$ 5.51	22.29 $\pm$ 7.89 ^{b**}		
BMI (kg/m ²)	26.61 $\pm$ 1.99	26.62 $\pm$ 1.51	27.22 $\pm$ 2.02	1.55
Albumin (g/dl)	4.12 $\pm$ 0.21	4.16 $\pm$ 0.33	4.23 $\pm$ 0.37	1.32
ALT (U/l)	19.17 $\pm$ 11.10	22.48 $\pm$ 13.33 ^{a*}	16.78 $\pm$ 4.71	3.59
AST (U/l)	31.83 $\pm$ 17.28 ^{a**}	31.21 $\pm$ 19.52 ^{a**}	16.06 $\pm$ 3.68	15.11
ALP (U/l)	194.78 $\pm$ 53.05	184.27 $\pm$ 55.72	169.24 $\pm$ 33.36	2.87
GGT (U/l)	125.91 $\pm$ 85.41 ^{a**}	74.84 $\pm$ 62.47 ^{a**b**}	21.49 $\pm$ 8.22	31.24
Bilirubin (mg/dl)	0.83 $\pm$ 0.23	0.72 $\pm$ 0.21 ^{b*}	0.74 $\pm$ 0.14	3.77
MMP-9 (ng/ml)	147.67 $\pm$ 35.89 ^{a**}	134.25 $\pm$ 36.14 ^{a**}	49.76 $\pm$ 11.45	135.65
IgG (mg/dl)	1287.19 $\pm$ 458.42 ^{a**}	1471.98 $\pm$ 334.13 ^{a**b*}	957.80 $\pm$ 227.9	27.13
IgE (IU/ml)	259.73 $\pm$ 222.58 ^{a**}	203.2 $\pm$ 130.79 ^{a**}	69.07 $\pm$ 22.061	19.85

Data were presented as mean $\pm$ SD.

^aOne way analysis of variances (ANOVA).

^a P value as compared to control group.

^b P value as compared to silicotic group.

* P value<0.05, ** P value<0.001.

Table 5A Pearson correlation in the exposed workers (N=87) measured parameters with age, smoking index, body mass index (BMI), and duration of work

		Albumin	ALT	AST	ALP	GGT	Bilirubin	MMP-9	IgG	IgE
Age	<i>r</i>	-0.044	0.049	0.174	-0.152	0.160	0.049	0.406**	0.146	0.096
	<i>P</i> value	0.684	0.655	0.106	0.161	0.138	0.649	0.000	0.177	0.374
Smoking Index	<i>r</i>	-0.036	0.321**	0.188	-0.099	-0.100	-0.161	-0.349**	0.075	-0.147
	<i>P</i> value	0.739	0.002	0.082	0.360	0.357	0.135	0.001	0.489	0.174
BMI	<i>r</i>	0.059	0.077	-0.052	-0.146	-0.209	-0.188	0.143	0.088	0.161
	<i>P</i> value	0.585	0.476	0.630	0.177	0.052	0.082	0.185	0.420	0.136
Duration of employment	<i>r</i>	-0.220*	0.117	0.23*	-0.20	0.14	0.08	0.356**	0.174	0.073
	<i>P</i> value	0.04	0.28	0.034	0.06	0.196	0.439	0.001	0.106	0.500

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

Analyses revealed a positive correlation between MMP9 and GGT ($P=0.037$) and a negative correlation between IgG, IgE, and GGT ($P<0.001$). In the silicotic workers, Pearson correlation revealed a significant positive correlation between MMP-9 ($P<0.001$) and IgG ($P=0.012$) with the disability rating for silicosis.

Next, exposed clay brick factory workers were classified by job type: miners ($n=27$) and production workers ($n=60$). The effect of occupational task and type on liver functions was investigated using a binary logistic regression model (Table 6). In this model the exposed workers (production and miners) were the dependent variable and MMP-9 and abnormal liver functions (ALT, AST, ALP, GGT, and Bilirubin) were the independent variables. After

adjusting for age and BMI, abnormal GGT and ALT were found to be associated with production workers (statistically significant for GGT). Abnormal GGT in production workers was approximately six times higher (Exp (B)=6.083, 95% CI=1.498–24.69) than in miners. Increased level of MMP-9 was one time higher in production workers than in miners (Exp (B)=1.02, 95% CI=1.00–1.04, $P<0.05$)

Discussion

Occupational exposure to crystalline silica in our study increased the risk of silicosis in the exposed workers who showed significant elevation in liver enzymes. Evidence of disturbed ECM remodeling was detected by increased level of MMP-9 associated with immunological disturbance.

Results indicated that brick factory employees involved in both mining and production activities are exposed to dust, smoke, and pollutant gases from the burning of biomass fuels.⁴ The mean levels of the total suspended and respirable dust did not exceed the limit standards of 10 and 3 mg/m³ respectively, mandated in Egyptian Environmental Law no. 4/ 1994.²⁹ However, the existence of free silica and different fumes might decrease markedly total dust threshold limit values (TLV), computed from the following formula:

$$\text{TLV of total dust (mg/m}^3\text{)} = \frac{30}{\text{Average free silica}\% + 3}$$

Table 5B Pearson correlation in the exposed workers (N=87) matrix metalloproteinase-9 (MMP-9) and immunoglobulins with liver functions

		MMP-9	IgG	IgE
albumin	<i>r</i>	-0.045	-0.073	0.082
	<i>P</i> value	0.676	0.500	0.452
ALT	<i>r</i>	0.118	-0.172	-0.127
	<i>P</i> value	0.278	0.112	0.240
AST	<i>r</i>	0.005	0.167	0.065
	<i>P</i> value	0.962	0.122	0.550
ALP	<i>r</i>	0.114	-0.111	-0.090
	<i>P</i> value	0.295	0.304	0.409
GGT	<i>r</i>	0.224(*)	-0.399**	-0.298**
	<i>P</i> value	0.037	0.000	0.005
Bilirubin	<i>r</i>	0.061	-0.247*	-0.170
	<i>P</i> value	0.573	0.021	0.115

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

Table 6 Logistic regression analysis for exposed workers

Independent variables	B	SE	Wald	Sig.	Exp (B)	95% CI
MMP-9	0.019	0.009	4.478	0.034	1.020	1.001–1.038
Abnormal ALT	0.404	0.790	0.261	0.609	1.498	0.318–7.047
Abnormal AST	-3.173	0.935	11.506	0.001	0.042	0.007–0.262
Abnormal ALP	-1.293	1.123	1.324	0.250	0.275	0.030–2.482
Abnormal GGT	1.805	0.715	6.379	0.012	6.083	1.498–24.695
Abnormal bilirubin	-2.686	1.693	2.519	0.112	0.068	0.002–1.880
Constant	-0.365	1.134	0.104	0.748	0.694	

The model $\chi^2=34.012$; $P<0.001$, overall percentage 82.9%.

-2 Log likelihood=73.760.

The dependant variable in this analysis was exposed worker. The independent variables were abnormal ALT, AST, ALP, GGT, and bilirubin.

The mean level of free crystalline silica in both sampled areas was approximately three-fold higher than the limit set by NIOSH of $50 \mu\text{g}/\text{m}^3$ and ACGIH of $25 \mu\text{g}/\text{m}^3$ for an 8-hour time-weighted average-minute period with an “R” notation. The “R” means that the sampling must be for “respirable particles.” Inhalation of respirable particles involves exposure to the particles in mineral dust that are able to penetrate into the alveolar spaces of the lungs. Generally, respirable particles have an aerodynamic diameter of $<3\text{--}4 \mu\text{m}$, while most particles larger than $5 \mu\text{m}$ will be deposited in the tracheobronchial airways and not reach the alveolar region. Particles deposited in the respiratory bronchioles and proximal alveoli are cleared more slowly and are more likely to injure the lung.^{30,31} Our findings emphasize the high risk for silicosis in the exposed workers. Overall, more than half of the exposed workers in this study (45/87, 51.7%) had abnormal radiological findings and 41.3% of the silica-exposed workers were diagnosed with silicosis.

The major determinant of silicosis is the lung total dust burden. Workers exposed to high concentrations of free crystalline silica in unprotected settings may be at risk for developing pulmonary fibrosis. The prevalence of the disease is dose-related with interaction of several factors including freshly fractured silica, admixtures of other minerals, and individual susceptibility.⁶

The prevalence of silicosis in miners was 40.7% (11/27) and 41.6% in production workers (25/60), showing no significant difference ($\chi^2=0.93$, OR [mining vs production] 0.974, 95% CI=0.514–1.844). Liou *et al.* found that workers in production lines had a higher prevalence of pneumoconiosis and pulmonary functions defects compared to other workers. They hypothesized that these findings were related to higher exposure to free silica.³² Along with smoking, occupational risk factors are a major cause of chronic respiratory illnesses and account for 13% of COPD, 11% of asthma, and almost all cases of silicosis, asbestosis, and pneumoconiosis worldwide.³³

The transport of silica particles via lympho-hematogenous routes after inhalation can result in their widespread dissemination. Silicotic lesions derived from thoracic silicosis may spread to the liver, spleen, bone marrow, and extrathoracic lymph nodes. The morphologic features of these lesions depend on the extent of macrophage aggregation, the occurrence of fibrogenesis, and the development of necrosis or degradation changes in ECM, presumably caused by lysosomal enzymes released from macrophages.^{34,35}

Accurate assessment of hepatic functional reserve is indispensable for the evaluation and selection of adequate treatments for patients with liver diseases. Several tests have been proposed for determining

residual liver function; however, no single marker is entirely reliable for predicting residual function, since hepatocytes possess a wide array of different functions. Instead of using a single marker, scoring systems using several parameters have been developed for assessing hepatic functional reserve and stratifying the severity of liver diseases. The evaluation systems work on the assumption that every parameter worsens in parallel according to the progression of liver disease, irrespective of the etiology.³⁶ We have detected impairment in liver function in workers exposed to silica dust marked by a statistically significant elevation of liver enzymes compared to the unexposed controls. More than half of the exposed workers had abnormal AST and GGT and almost 20% had abnormal ALT and ALP levels. Serum albumin level was within the normal range for all study participants. However, there was a statistically significant decline in albumin levels with increased work duration, reflecting a parallel decline in the hepatic capacity of protein synthesis.

Other researchers have reported significant disturbance of some liver function tests in silica-exposed workers.^{37–39} In contrast Mojiminiyi *et al.* found no significant changes in liver functions among a study of cement workers in Nigeria.⁴⁰

In an experimental study with 2-month-old male CBA mice, silica exposure was associated with extensive and early development of destructive fibrotic processes in the liver parenchyma and depression of cellular and intracellular reparative regeneration.⁷

Previous studies have proposed several mechanisms for possible liver affection in silica-exposed workers including: immunological disturbance, ECM remodeling, oxidative/inflammatory pathway, and the epithelial mesenchymal transition theory.^{11,41,42}

Altered MMPs activity, by either the higher expression of the enzymes or their inhibitors, may have different effects on lung matrix remodeling. Gelatinases A (MMP-2) and B (MMP-9) up regulated early in the lung parenchyma of silicotic rats, resulting in basement membrane disruption, an important pathological event observed in lung lesions that evolve into fibrosis.⁴³ In our study, over expression of MMP-9 was found in the exposed workers with a statistically significant difference compared with the unexposed controls ($P<0.001$). This indicates that the balance in the normal ECM in silica-exposed workers was disturbed and went toward the side of degradation with the possible role of MMP-9. This conclusion was further confirmed by the significant positive correlation between the level of MMP-9 and duration of employment in the exposed workers ($r=0.356$, $P<0.001$).

In an experimental study, Perez-Ramos *et al.* found marked expression of collagenase 3, and gelatinases

A and B in early silica exposure which were apparent not only inside granulomas, but also in collections of macrophages situated in the alveoli and interstitial tissues around the respiratory bronchioles.⁴⁴ Interestingly, MMPs expression had decreased after 60 days of exposure, mainly in fibrotic granulomas. In a similar experimental study, Scabilloni *et al.* found overexpression of MMP-2 and MMP-9, but contrary to Perez-Ramos study, this initial increase continued with increased exposure time.¹⁴

If we relate these findings to our study, workers with established silicosis showed no significant difference in the mean level of the enzyme compared to workers with no silicosis. We found no significant correlation between MMP-9 and duration of employment in workers with silicosis ($r=0.224$, $P=0.189$). Chronic silicosis results from recurring exposure to relatively low levels of silica and is characterized by silicotic nodules in the worker's lungs.⁴⁵ Our findings indicate that MMP-9 is over expressed early in the exposure to silica and that the overexpression did not continue after the development of chronic silicosis evidenced by the appearance of silicotic nodules.

One aspect of nodule development is the connective tissue remodeling that occurs at the core of these lesions. Remodeling, a normal event that continually occurs in healthy lungs, is a process whereby ECM is recycled. However, an aberrant remodeling process occurs as a result of damage to the ECM from silica inhalation, and the balance seen in the normal lungs no longer exists.⁴¹

Degradation of normal liver matrix may contribute to the pathogenesis of liver disease, particularly in the early stages of liver injury response. Evaluation of fibroproliferative activity is difficult and liver biopsy is still regarded as the gold standard for evaluation. Several biochemical indicators have been discussed as potential non-invasive markers of fibroproliferation. For example, the MMPs and their tissue inhibitors (TIMPs) are correlated with the development of liver cirrhosis, toxic liver damage, and the inflammatory activity in chronic viral hepatitis.^{46,47} Matrix metalloproteinase-9 may be especially important for the development of organ injury because they degrade type IV (basal membrane) collagen and thus are involved in the early stages of tissue remodeling that characterizes chronic liver diseases.⁴⁸

A significant positive correlation was found between MMP-9 and GGT in the exposed group, and correlation was positive in the exposed subgroups (but significant only in the non-silicotic workers). This may indicate a possible role of MMP-9 in liver affection in silica-exposed workers. Moreover, the findings of the binary logistic regression analysis showed that MMP-9, abnormal GGT, and ALT were associated with working in clay brick production

(significant for GGT), indicating that production workers were at a higher risk for impaired liver functions and the possible role for MMP-9. This may be explained by the fact that production workers are additionally exposed to chemical hepatotoxins in their occupational settings that may add stress to the liver.⁴

More than 40 years ago, Vigliani and Pernis reported extensive serological and cellular evidence of activation of the immune system in human and experimental silicosis.⁴⁹ Recently, silica is considered to act as a PAMP (pathogen-associated molecular pattern) with the receptors of the key cells of innate immunity (the macrophages). This causes initial stimulation and then death of macrophages, which release cytokines (IL-1 and TNF) that stimulate fibroblasts. In view of these facts, silicosis can be classified as a "collagen disease."¹¹

In this study, a highly significant difference was found between the exposed and control groups in the mean levels of IgG and IgE ($P<0.001$). Anti-liver kidney microsomal antibody was not detected in any of the studied subjects. These findings are in agreement with previous studies that have found increased levels of immunoglobulins in silica-exposed workers.^{9,50}

The theory is that exposure to silica will initially suppress humoral immune system, while chronic exposure to silica (and silicosis) will stimulate the immunity.¹¹ The exposed workers in this study had a minimum of 5 years of chronic exposure to silica dust and almost half had silicosis. Stimulation of the immune system was expected.

The correlation of immunoglobulins with duration of employment showed a positive but non-significant correlation ($P>0.05$). The correlations of immunoglobulins with liver functions parameters were also non-significant, except for GGT and bilirubin, where there was a significant negative correlation. These findings, coupled with no detectable anti-liver kidney microsomal antibodies, led us to conclude a questionable role of immunological factors in liver affection in our studied population.

In this study clay brick workers showed disturbance of liver functions, which was more marked in production workers. Matrix remodeling may be regarded as an important pathway in the pathogenesis of this effect. Although immunological factors had a questionable role in liver affection in this study, it should be mentioned that the immunological aspect was limited and numerous immune pathways exist that could not be ruled out. Our study indicated that silica-induced liver disease may contribute to morbidity in silica-exposed workers. Although additional research is needed to characterize the risks among other types of occupational exposures to silica, we think that liver functions should be monitored among

silica-exposed workers and should be taken into account during therapy of patients with silicosis and silicotuberculosis implying the use of hepatotoxic antituberculous drugs.

One limitation of this study was the small sample sizes of the groups, limiting the possibility of an efficient exposure–response analysis. However, we were able to control for cofounders to some extent through sample selection. Thus the observed exposure–response relationships presumably reflect the effects of occupational dust/silica exposure. Large-scale studies and other industrial activities where mixed exposure with complex interaction of silica and hepatotoxic chemicals occurs are needed.

Finally, to reduce the adverse effects of industrial silica exposure, it is important to evaluate the degree, type, and source of exposure. Optimally, silica-containing materials should be replaced; work processes should be isolated and enclosed; adequate ventilation should be provided; and personal protective equipment used at all times during possible silica exposure. Even with such measures, some settings may witness rates of exposure that exceed guidelines. Consequently, it is clear that continued efforts are needed to train and supervise workers to promote worker safety with regard to silica exposure. Brick production plants in Egypt are mostly owned privately and the National Environmental Protection Agency and policy makers should impose and enforce stricter regulations to control silica dust exposure.

Disclaimer statements

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Ethics approval All the included subjects were treated according to the Helsinki Declaration of biomedical ethics. Informed consent was obtained from the participants after proper orientation regarding the objectives of the study, and the data confidentiality. The approval of the research ethics committee of Faculty of Medicine, was taken and the workers were given a report of their investigations. Findings and recommendations were delivered to the factory EHS administration.

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