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Others

Assessment of Diastolic Function and Thiol-Disulphide Homeostasis in Arsenic-Exposed Workers

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Objectives: Exposure to arsenic is associated with various cardiovascular diseases. The imbalance between antioxidant and oxidant homeostasis plays a crucial role in the cardiovascular effects of arsenic. The aim of this study was to investigate the effect of arsenic exposure on diastolic function by measuring thiol and disulphide in arsenic-exposed workers.

Methods and Results: A total of 107 male arsenic-exposed workers and 36 healthy subjects were enrolled. Mitral inflow velocity and parameters of diastolic function were measured. As oxidative stress indicators, total thiol, native thiol, disulphide, and their percent ratios were determined. The mean age was 39.1 ± 9.5 years in the arsenic-exposed group and 37.4 ± 9.6 years in the controls. The median blood arsenic level was 42 μg/dL in the arsenic-exposed group and 3.75 μg/dL in the controls. E-wave, E/A ratio, and e′ wave were lower and left atrial diameter, A-wave, average E/e′ ratio, and tricuspid regurgitation velocity were higher in the arsenic-exposed group. Native and total thiol concentrations were lower, and disulphide/native and disulphide/total thiol ratios were higher in the arsenic-exposed group. Fourteen (13.1%) workers had diastolic dysfunction, 26 (24.3%) had indeterminate, and 67 (62.6%) had normal diastolic function, compared to 1 (2.8%), 2 (5.6%), and 33 (97.7%) in the control group, respectively. In regression analysis, disulphide/native thiol ratio ($p < 0.001$) and blood arsenic level ($p < 0.001$) predicted increased average E/e′ ratio in the arsenic-exposed group.

Conclusions: This study showed strong associations among arsenic exposure, oxidative stress, and diastolic function, and revealed the influence of arsenic exposure on diastolic dysfunction through oxidative stress.

Key Words: Arsenic • Diastolic function • Disulphide • Oxidative stress • Thiol

INTRODUCTION

Environmental and occupational exposure to arsenic is associated with various and harmful cardiovascular disorders including coronary heart disease, left ventricular (LV) hypertrophy, stroke, hypertension, and increased cardiovascular mortality.[1-5] Several studies have shown arsenic-induced impairment in antioxidant and oxidant balance due to excessive reactive oxygen species (ROS) production, and that increased oxidative stress plays a crucial role in cardiac damage.[6-8] Hence, these changes may lead to worsening of systolic and diastolic functions.[9-11] For instance, a study by Dai et al. demon-

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strated that ROS production triggered maladaptive remodeling resulting in diastolic dysfunction.[12]

Thiol-containing biological substances have been shown to have stronger antioxidant properties compared to other antioxidant substances such as superoxide dismutase, catalase, vitamin A, C, and E in recent years. Thiols are organic substances consisting of sulphur, hydrogen and carbon, and prevent tissues from oxidative stress by interacting with ROS with their –SH group.[13] Thiol molecules lose their hydrogen with oxidation, converting to their reversible form of disulphide (-S-S-). This cycle between thiol and disulphide represents the basis of antioxidant and oxidant homeostasis. Therefore, the adverse effects of oxidative stress can be assessed by measuring thiol and disulphide homeostasis.[14]

In the present study, we aimed to investigate the associations among arsenic exposure, diastolic function, and thiol-disulphide homeostasis in arsenic-exposed workers compared to healthy subjects.

MATERIALS AND METHODS

Study population

This cross-sectional and observational study enrolled a total of 107 male workers occupationally exposed to arsenic who were referred to our clinic for high blood arsenic levels (> 15 μg/dL). In addition, 36 healthy subjects without known cardiovascular diseases were enrolled in the study as controls. The arsenic exposure group consisted of subjects working in jobs that involved arsenic production or use, such as copper or lead smelting, wood treatment, or pesticide application. Medical history and physical examination findings including height (m), weight (kg), body mass index calculated as weight/height^2 and blood pressure (BP) were recorded on the day of transthoracic echocardiography (TTE). Twelve-lead surface electrocardiogram, laboratory, and TTE findings were recorded. Subjects previously diagnosed with coronary artery disease, hypertension, diabetes mellitus, thyroid dysfunction, hyperlipidemia, smokers, and chronic lung disease were excluded. Also excluded were those with sinus tachycardia, first degree atrioventricular block, and atrial fibrillation which affected the mitral inflow pattern. All participants were male. As a standard of care/action in our medical center, it was confirmed based on patient records that all of the study participants gave informed consent at the time of hospitalization and relevant diagnostic/therapeutic standards of care. All of the participants' records/information were anonymized prior to analysis.

Transthoracic echocardiography

Standard TTE imaging was performed according to the recommendations of the American Society of Echocardiography.[15] Images were obtained using a 2.5-3.5 MHz transducer in the left lateral decubitus position. LV end-diastolic and end-systolic diameters were estimated with M-mode echocardiography in the parasternal long-axis view. LV ejection fraction was calculated in the apical 4-chamber view, according to modified Simpson's rule. Left atrial (LA) volume was obtained using the biplane area-length method from apical 4- and 2-chamber views at end-systole just prior to mitral valve opening, and was corrected for body surface area and recorded in mL/m^2 (LA volume index). LV mass was calculated using the Devereux formula and normalized by body surface area [LV mass index (LVMI)].[16] Mitral valve inflow velocities including peak early filling (E wave) and late diastolic filling (A wave) velocities, E/A ratio, deceleration time, and isovolumic relaxation time were recorded in the apical four-chamber view using pulsed-wave Doppler with the Doppler beam aligned parallel to the direction of flow and sample volume positioned at the tips of the mitral leaflets. Tissue Doppler imaging of the mitral annulus was obtained by positioning a 1-3 mm sample volume at the lateral hinge point of the mitral valve in a four-chamber view. Gains were adjusted to eliminate noise for clear tissue signal. Doppler signals were recorded at a paper speed of 100 mm/s. The peak early diastolic tissue velocities (lateral and septal e' waves) were averaged from three end-expiratory cycles. Average E/e' ratio, as a surrogate marker of LV filling pressure, was calculated, and a value greater than 14 was regarded as a predictor of increased LV filling pressure.[17,18] Continuous-wave Doppler in the apical four-chamber view was used to obtain the peak velocity of tricuspid regurgitation (TR) with a sweep speed of 100 mm/s to achieve a satisfactory envelope.

The evaluation of LV diastolic function was performed according to the 2016 recommendations of the American Society of Echocardiography and the European As-

sociation of Cardiovascular Imaging.[17] The following four variables were used to identify diastolic dysfunction:

1. Mitral lateral e' wave < 10 cm/sec
2. Average E/e' ratio > 14
3. LA volume index > 34 mL/m^2
4. Peak TR velocity > 2.8 m/sec

The subjects were identified as having diastolic dysfunction if more than half of the four variables met the cut-off values. Subjects were identified as having indeterminate diastolic function if two variables met the cut-off values, and normal diastolic function if more than half of the variables did not meet the cut-off values.

Biochemical tests and analysis

Whole blood arsenic levels, taken from the workers at the end of a work shift, were analysed using Inductively Coupled Plasma Mass Spectrometry (Agilent 7700 series, Tokyo, Japan). Blood samples were digested using the microwave-induced acid digestion method. A standard solution of arsenic was prepared by dilution of certified standard solutions (High purity Standards, Charleston, SC, USA). Two levels of quality control materials were used (Seronorm, Billingstad, Norway). For serum disulphide and thiol homeostasis measurements, we used the spectrophotometric method described by Erel and Neselioglu.[19] Briefly, reducible disulphide bonds were reduced to form free functional thiol groups. Unused reductant sodium borohydride was consumed and removed with formaldehyde, and all thiol groups including reduced and native thiol groups were determined after the reaction with 5,5′-dithiobis-(2-nitrobenzoic) acid. Half of the difference between total thiols and native thiols was recorded as the amount of dynamic disulphide. After native thiols and total thiols had been determined, the amount of disulphide, disulphide/total thiol percent ratios, disulphide/native thiol percent ratios, and native thiol/total thiol percent ratios were calculated.

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0; IBM SPSS Inc., Chicago, IL). Variables with normal distribution were presented as mean ± standard deviation, while those without normal distribution were presented as median and interquartile range (IQR, 25th and 75th percentiles). For determining whether a variable showed normal distribution, Kolmogorov-Smirnov and Shapiro-Wilk tests were used. In addition, skewness and kurtosis values of variables were controlled. Categorical variables were presented as number and percentage. Comparisons of continuous variables were performed using the t-test for independent variables and one-way ANOVA with normal distribution, Mann-Whitney U and Kruskal-Wallis tests for those without normal distribution, and the chi-square test for categorical variables. The correlations between the variables with normal distribution were analysed using Pearson correlation, and Spearman correlation analysis was used for variables without normal distribution. According to the assumption of normality, logarithmic transformation was applied to parameters that did not show normal distribution. Univariate linear regression analysis was performed for each potential risk variable. As independent variables, baseline demographic, clinical, and laboratory characteristics with a p value of < 0.1 in univariate analysis were included into multivariate regression analysis with a "stepwise" model to predict E/e' ratio. In the multivariate regression analysis, tolerance and variance inflation factor values of collinearity statistics were taken into consideration to avoid potential strong correlations among the independent variables. Receiver operating characteristic (ROC) curve analysis was performed to analyse the diagnostic value of blood arsenic level and disulphide/thiol ratio and to identify the optimal cut-off values of these variables to predict increased LV end-diastolic pressure (average E/e' ratio < 14 or not). Further ROC curve analysis was performed to determine the diagnostic value of blood arsenic level and disulphide/thiol ratio in diastolic dysfunction in the arsenic-exposed workers. A p value of < 0.05 was considered to be statistically significant.

RESULTS

Baseline demographic, clinical, and laboratory characteristics of the arsenic-exposed and control groups are presented in Table 1. No statistically significant differences were found between the groups in age, resting heart rate, diastolic BP, body mass index, and baseline biochemical and blood count findings. The mean age of

the arsenic-exposed group was 39.1 ± 9.5 years, and that of the control group was 37.4 ± 9.6 years (p = 0.349). Systolic BP was higher in the arsenic-exposed group compared to the controls (122.9 ± 9.3 mmHg vs. 118.7 ± 10.3 mmHg, p = 0.025). Diastolic BP was also higher in the arsenic-exposed group, but this difference did not reach statistical significance. The median blood arsenic level in the arsenic-exposed group was 42 μg/dL (IQR; 28.8-63), which was significantly higher than that of the controls (3.75 μg/dL, IQR; 2.82-6.15, p < 0.001). Thiol and disulphide concentrations and disulphide/native thiol and disulphide/total thiol ratios are also presented in Table 1. While native and total thiol concentrations were significantly lower (Figure 1A and B respectively), disulphide/native thiol ratio and disulphide/total thiol ratio were significantly higher in the arsenic-

Table 1. Baseline demographic, clinical, and laboratory characteristics of the arsenic-exposed and control groups

Characteristics	Control group (n = 36)	Arsenic-exposed group (n = 107)	p value
Age (years)	37.4 ± 9.6	39.1 ± 9.5	0.349
Resting heart rate (bpm)	71.8 ± 2.6	75.3 ± 13.9	0.183
Systolic blood pressure (mmHg)	118.7 ± 10.3	122.9 ± 9.3	0.025
Diastolic blood pressure (mmHg)	72.3 ± 7.0	74.0 ± 7.1	0.214
Body mass index (kg/m^2)	24.6 ± 5.7	22.7 ± 6.1	0.103
White blood cell count (/μL)	7071 ± 1504	7172 ± 1575	0.730
Hemoglobin (g/dL)	14.9 ± 1.2	14.8 ± 1.6	0.698
Platelet count (/μL)	237610 ± 49860	237500 ± 43026	0.991
Creatinine (mg/dL)	0.90 ± 0.12	0.88 ± 0.16	0.698
Alanine aminotransferase (U/L)	24 [17.75-32.5]	25 [18-34]	0.950
Aspartate aminotransferase (U/L)	20.5 [18.25-22]	21 [17-24]	0.944
High-density lipoprotein (mg/dL)	48.7 ± 10.8	45.4 ± 10.3	0.110
Low-density lipoprotein (mg/dL)	113.3 ± 28.6	107.4 ± 30.0	0.294
Triglyceride (mg/dL)	140.5 [99.75-173.25]	140.0 [90-197]	0.983
Total cholesterol (mg/dL)	189.6 ± 40.5	180.6 ± 35.0	0.241
Blood arsenic level (μg/dL)	3.75 [2.82-6.15]	42 [28.8-63]	< 0.001
Native thiol (μmol/L)	433.8 ± 43.6	408.2 ± 56.2	0.014
Total thiol (μmol/L)	467.7 ± 44.4	445.3 ± 56.0	0.031
Disulphide (μmol/L)	16.9 ± 4.5	18.5 ± 4.9	0.088
Disulphide/native thiol ratio (%)	3.94 ± 1.15	4.65 ± 1.48	0.010
Disulphide/total thiol ratio (%)	3.64 ± 0.97	4.22 ± 1.23	0.010

Variables are expressed as mean ± standard deviation or median and [25th-75th percentile].

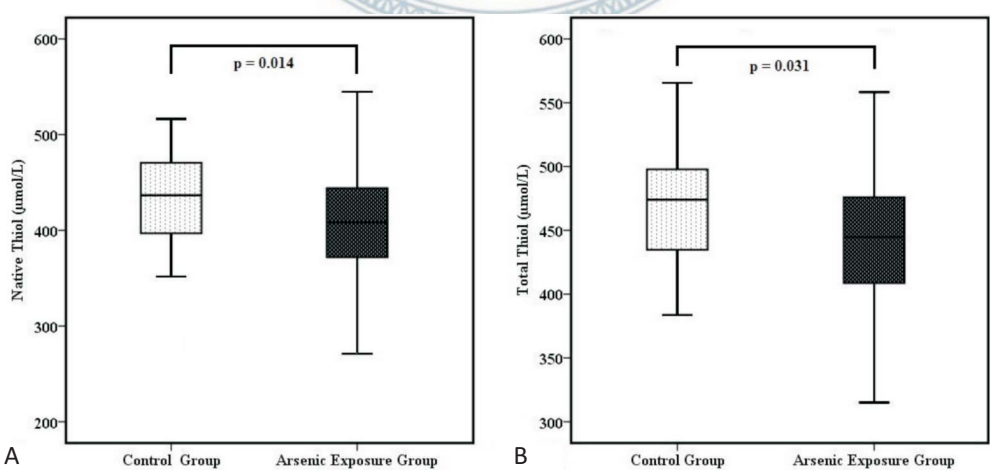

Figure 1. *Thiol concentrations in the arsenic-exposed and control groups. Native (A) and total (B) thiol concentrations were lower in the arsenic-exposed group than the control group. The decrease in both thiols indicates an impaired antioxidant capacity.*

exposed group compared to the controls. The disulphide concentration was also higher in the arsenic-exposed group, but this difference did not reach statistical significance.

Transthoracic echocardiographic parameters of the two groups are presented in Table 2. LV end-diastolic and end-systolic diameters and ejection fraction were similar in both groups. E-wave, E/A ratio, and lateral (Figure 2A) and septal e′ waves were lower in the arsenic-exposed group compared to the controls. In con-

Table 2. Transthoracic echocardiographic parameters of the arsenic-exposed and control groups

Parameters	Control group (n = 36)	Arsenic-exposed group (n = 107)	p
LVEDD (mm)	46.6 ± 3.7	46.0 ± 2.7	0.316
LVESD (mm)	28.7 ± 3.9	28.4 ± 2.8	0.698
LV ejection fraction (%)	64.0 ± 3.3	64.6 ± 3.4	0.365
LV mass index (g/m^2)	77.0 ± 16.6	79.5 ± 12.6	0.415
LA diameter (mm)	32.2 ± 2.1	33.7 ± 3.8	0.049
E-wave (cm/sec)	111.5 ± 8.1	104.0 ± 11.6	0.001
A-wave (cm/sec)	85.9 ± 10.8	102.8 ± 15.4	0.001
E/A ratio	1.31 ± 0.20	1.03 ± 0.18	0.001
Lateral e′ wave (cm/sec)	13.2 ± 1.0	10.7 ± 1.8	0.001
Septal e′ wave (cm/sec)	9.2 ± 1.3	7.0 ± 1.7	0.001
Average E/e′ ratio	10.4 ± 1.4	12.8 ± 2.9	0.001
Isovolumic relaxation time (msec)	80.6 ± 6.7	83.2 ± 7.9	0.077
Deceleration time (msec)	178.6 ± 15.3	183.5 ± 14.7	0.090
LA volume index (mL/m^2)	28.7 ± 2.1	29.3 ± 3.0	0.220
TR velocity (cm/sec)	2.63 ± 0.20	2.73 ± 0.26	0.016
Diastolic function			0.032
Normal	33 (97.7)	67 (62.6)	0.001
Indeterminate	2 (5.6)	26 (24.3)	
Dysfunction	1 (2.8)	14 (13.1)	

LA, left atrium; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; TR, tricuspid regurgitation.
Variables are expressed as mean ± standard deviation or number (percentage).

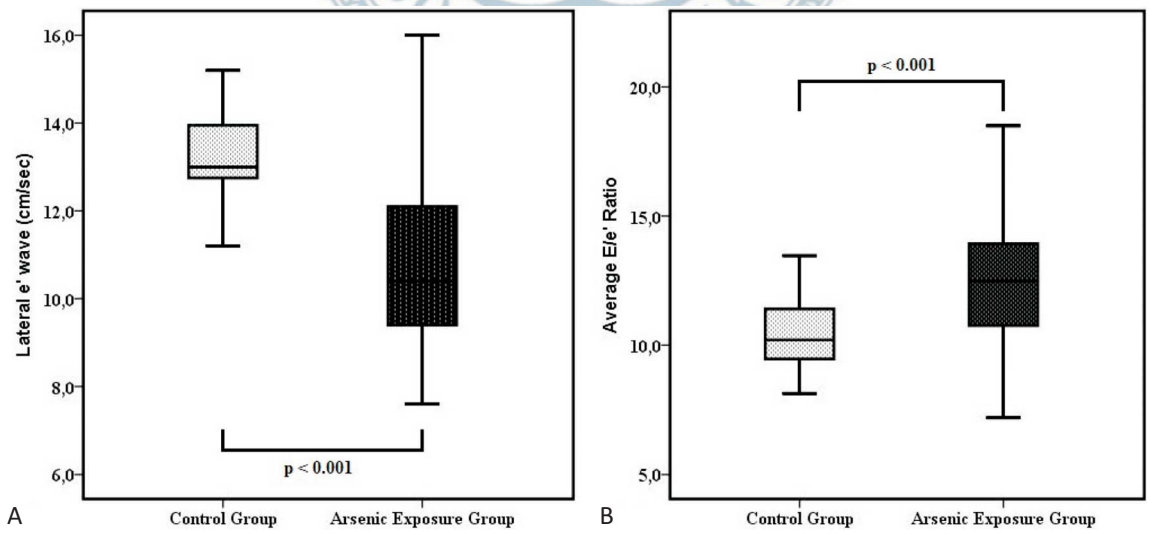

Figure 2. *Lateral e' wave and average E/e' ratio in the arsenic-exposed and control groups. Lateral e' wave was lower (A) and average E/e' ratio was higher (B) in the arsenic-exposed group than the control group. These indicate worsening myocardial relaxation and diastolic dysfunction in arsenic-exposed workers.*

trast, LA diameter, A-wave, average E/e′ ratio (Figure 2B), and peak TR velocity were higher in the arsenic-exposed group compared to the controls. LVMI, LA volume index, isovolumic relaxation time, and deceleration time were similar. According to the 2016 recommendations of the American Society of Echocardiography and European Association of Cardiovascular Imaging, of 107 arsenic-exposed workers, 14 (13.1%) had diastolic dysfunction, 26 (24.3%) had an indeterminate function, and 67 (62.1%) had a normal diastolic function, compared to1 (2.8%), 2 (5.6%), and 33 (97.7%) in the control group (n = 36), respectively. The difference between the groups was significant (p = 0.001) (Figure 3).

Table 3 lists the baseline demographic, clinical, and laboratory characteristics of the arsenic-exposed workers according to diastolic function status. In the workers with diastolic dysfunction, the blood arsenic level was significantly higher than in the normal and indeterminate groups (p = 0.001). Native and total thiol concentrations were lower in the workers with diastolic dysfunction than in those with normal or indeterminate diastolic function, although the differences were not statistically significant (p values 0.053 and 0.173, respectively). Additionally, disulphide concentration, disulphide/native thiol, and disulphide/total thiol ratios were significantly higher in the workers with diastolic dysfunction than in those with normal or indeterminate diastolic function (p values 0.007, 0.001, and 0.001, respectively). No obvious differences in age, systolic and diastolic BP, creatinine, and low-density lipoprotein cholesterol level were found among the groups.

Correlation analysis

Correlation analyses in the arsenic-exposed group are presented in Table 4. Blood arsenic level was negatively correlated with native and total thiol concentrations, and positively correlated with disulphide/native thiol and disulphide/total thiol ratios. However, there was no significant correlation between blood arsenic level and disulphide concentration (p = 0.059). In addition, blood arsenic level was positively correlated with

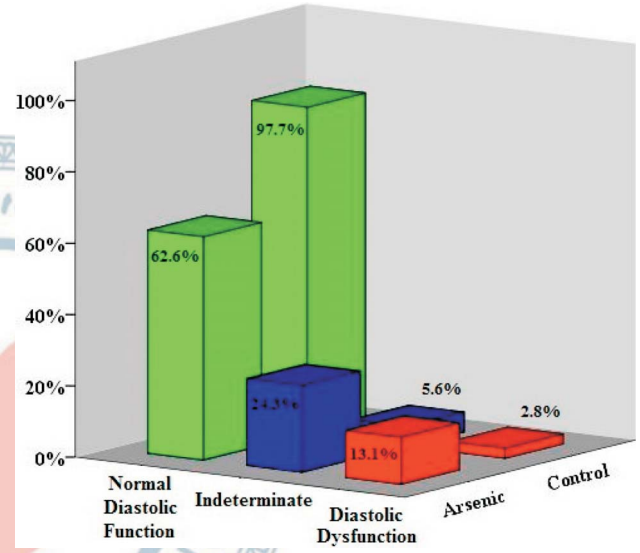

Figure 3. *Distribution of diastolic function status. The percentage of subjects with normal diastolic function, indeterminate, and diastolic dysfunction in arsenic-exposed and control groups.*

Table 3. Baseline demographic, clinical, and laboratory characteristics of the arsenic-exposed workers according to the status of diastolic function

Characteristics	Normal (n = 67)	Indeterminate (n = 26)	Diastolic dysfunction (n = 14)	p
Blood arsenic level (μg/dL)	35 [24-49]	44.9 [39-65]	82.5 [49-146]	0.001
Native thiol (μmol/L)	416.9 ± 53.1	401.4 ± 50.8	378.8 ± 71.0	0.053
Total thiol (μmol/L)	452.2 ± 53.8	439.7 ± 50.8	422.8 ± 70.9	0.173
Disulphide (μmol/L)	17.6 ± 4.9	19.1 ± 4.7	22.0 ± 4.2	0.007
Disulphide/native thiol (%)	4.2 ± 1.3	4.8 ± 1.4	6.0 ± 1.6	0.001
Disulphide/total thiol (%)	3.9 ± 1.1	4.4 ± 1.1	5.3 ± 1.3	0.001
Age (years)	38.9 ± 9.8	39.6 ± 8.7	39.4 ± 9.9	0.946
Systolic BP (mmHg)	123.6 ± 8.9	121.2 ± 10.8	123.0 ± 8.2	0.530
Diastolic BP (mmHg)	74.5 ± 7.1	73.7 ± 7.5	72.3 ± 6.8	0.569
Creatinine (mg/dL)	0.89 ± 0.16	0.87 ± 0.19	0.84 ± 0.11	0.517
LDL-C (mg/dL)	105.1 ± 29.2	119.3 ± 30.1	95.8 ± 28.4	0.036

BP, blood pressure; LDL-C, low density lipoprotein cholesterol.
Variables are expressed as mean ± standard deviation or median and [25^{th}-75^{th} percentile].

average E/e′ ratio (r = 0.484, p < 0.001). Average E/e′ ratio was negatively correlated with native and total thiol concentrations, but positively correlated with disulphide concentration, and disulphide/native thiol and disulphide/total thiol ratios.

Regression analysis

Univariate and multivariate regression analyses findings are presented in Table 5. As possible demographic, clinical, and laboratory risk factors for average E/e′ ratio, a large number of variables were analysed in the univariate regression analysis. Blood arsenic level, disulphide concentration, and disulphide/native thiol and disulphide/total thiol ratios were associated with an increased average E/e′ ratio. On the other hand, native and total thiol ratios were associated with a reduced average E/e′ ratio. The variables with p values < 0.1 in the univariate analysis along with systolic and diastolic BP and LVMI were entered into multivariate regression analysis. The results showed that blood arsenic level (β ± SE = 1.67 ± 0.41; p < 0.001) and disulphide/native thiol ratio (β ± SE = 0.64 ± 0.17; p < 0.001) were independently associated with increased average E/e′ ratio (model R^2 0.332, p < 0.001).

ROC curve analysis was performed to assess the power of blood arsenic level and disulphide/native thiol ratio for predicting increased LV diastolic pressure in the arsenic-exposed group (Figure 4A). The area under curve (AUC) was 0.749 [95% confidence interval (CI) 0.652-0.847, p < 0.001], and the sensitivity and specificity were 81% and 56%, respectively, at a cut-off point of 39.7 μg/dL for blood arsenic level. In addition, when the disulphide/native thiol ratio was 4.19, the AUC was 0.804 (95% CI 0.707-0.901, p < 0.001) and the sensitivity and specificity were 88% and 52%, respectively.

Further ROC curve analysis was performed to assess the power of blood arsenic level and disulphide/native

Table 4. Correlation analysis among blood arsenic level, thiol – disulphide concentrations, and average E/e′ ratio in the arsenic-exposed group

	Blood arsenic level		Average E/e′ ratio	
	r	p	r	p
Blood arsenic level (μg/dL)	-	-	0.484	< 0.001
Native thiol (μmol/L)	-0.321	0.001	-0.311	0.001
Total thiol (μmol/L)	-0.302	0.002	-0.248	0.010
Disulphide (μmol/L)	0.183	0.059	0.359	< 0.001
Disulphide/native thiol (%)	0.322	0.001	0.473	< 0.001
Disulphide/total thiol (%)	0.313	0.001	0.468	< 0.001

Table 5. Univariate and multivariate regression analyses findings of the possible risk factors for predicting average E/e′ ratio

Risk factors	Univariate regression analysis				Multivariate regression analysis			
	β ± SE	95% CI		p	β ± SE	95% CI		p
		Lower	Upper			Lower	Upper	
Blood arsenic level (μg/dL)	2.28 ± 0.39	1.516	3.06	< 0.001	1.67 ± 0.41	0.865	2.476	< 0.001
Native thiol (μmol/L)	-0.016 ± 0.005	-0.026	-0.007	< 0.001				
Total thiol (μmol/L)	-0.013 ± 0.005	-0.023	-0.003	0.010				
Disulphide (μmol/L)	0.214 ± 0.054	0.106	0.321	< 0.001				
Disulphide/native thiol ratio (%)	0.94 ± 0.17	0.601	1.279	< 0.001	0.64 ± 0.17	0.175	0.296	< 0.001
Disulphide/total thiol ratio (%)	1.12 ± 0.2	0.711	1.529	< 0.001				
Age (years)	0.016 ± 0.30	-0.044	0.076	0.599				
Left ventricle mass index (g/m^2)	-0.007 ± 0.023	-0.053	0.038	0.750				
Systolic blood pressure (mmHg)	-0.036 ± 0.31	-0.096	0.025	0.246				
Diastolic blood pressure (mmHg)	-0.07 ± 0.04	-0.152	0.008	0.077				
Hemoglobin (g/dL)	0.29 ± 0.17	-0.056	0.651	0.098				
Creatinine (mg/dL)	-1.78 ± 1.75	-5.27	1.67	0.307				
Low-density lipoprotein (mg/dL)	-0.001 ± 0.006	-0.013	0.012	0.936				
Aspartate aminotransferase (U/L)	0.90 ± 0.855	-0.797	2.62	0.295				
Alanine aminotransferase (U/L)	-0.09 ± 0.531	-1.156	0.961	0.857				
Triglyceride (mg/dL)	-0.74 ± 0.462	-1.662	0.178	0.112				

CI, confidence interval; SE, standard error.

thiol ratio for predicting diastolic dysfunction in the arsenic-exposed group (Figure 4B). The AUC was 0.837 (95% CI 0.739-0.935, p < 0.001), and the sensitivity and specificity were 85% and 68%, respectively, at a cut-off point of 45.3 μg/dL for blood arsenic level. For disulphide/native thiol ratio, the AUC was 0.762 (95% CI 0.616-0.908, p = 0.002), and the sensitivity and specificity were 79% and 64%, respectively, at a cut-off point of 4.83.

DISCUSSION

In this study, we demonstrated a deterioration in LV diastolic function and thiol-disulphide homeostasis in arsenic-exposed workers. In addition, there were significant correlations among parameters of diastolic function, blood arsenic levels, and thiol-disulphide concentrations in the arsenic-exposed workers. Blood arsenic levels and disulphide/native thiol ratio could independently predict the average E/e' ratio, which is an important echocardiographic parameter for diastolic function.

Arsenic is a common heavy metal which has many cardiotoxic effects. Mechanisms thought to mediate the cardiotoxic effects of arsenic include oxidative stress, DNA fragmentation, induction of apoptosis, and functional changes in ion channels.[8] Oxidative stress increases due to the increased production of various ROS in cases of arsenic exposure. Arsenic changes the oxidant-antioxidant balance in favor of oxidant mechanisms by interacting with antioxidant substances. In other words, arsenic does not only stimulate ROS production but also consumes antioxidant reserves. In the present study, total and native thiol concentrations, which indicate antioxidant reserve, were decreased, and disulphide concentration, which is an oxidant form of these thiols, was increased in the arsenic-exposed group compared to the controls. Consequently, our findings showed unfavorable effects of arsenic on between oxidant and antioxidant balance.

Glutathione is a source of thiol that facilitates the intercellular diffusion of arsenic by reacting with it and accelerating its excretion by converting it to methylated metabolites.[20] In an experimental study, Manna et al. showed that arsenic intoxication decreased cardiac glutathione and total thiol concentrations, and increased the levels of oxidized glutathione.[21] However, the published reports are mainly experimental, manual, sophisticated studies, and they generally relate to the determination of the amount of thiol and disulphide in low-molecular-weight thiol compounds such as glutathione,[22,23] thioredoxin reductase,[24] humic acid,[25] and γ-glutamylcysteine.[26] In the present study, the association between arsenic toxicity and oxidative stress in the arsenic-exposed workers was investigated using thiol/disulphide homeostasis by measuring mainly native thiol and reducible dynamic disulphide amounts using an easy, inex-

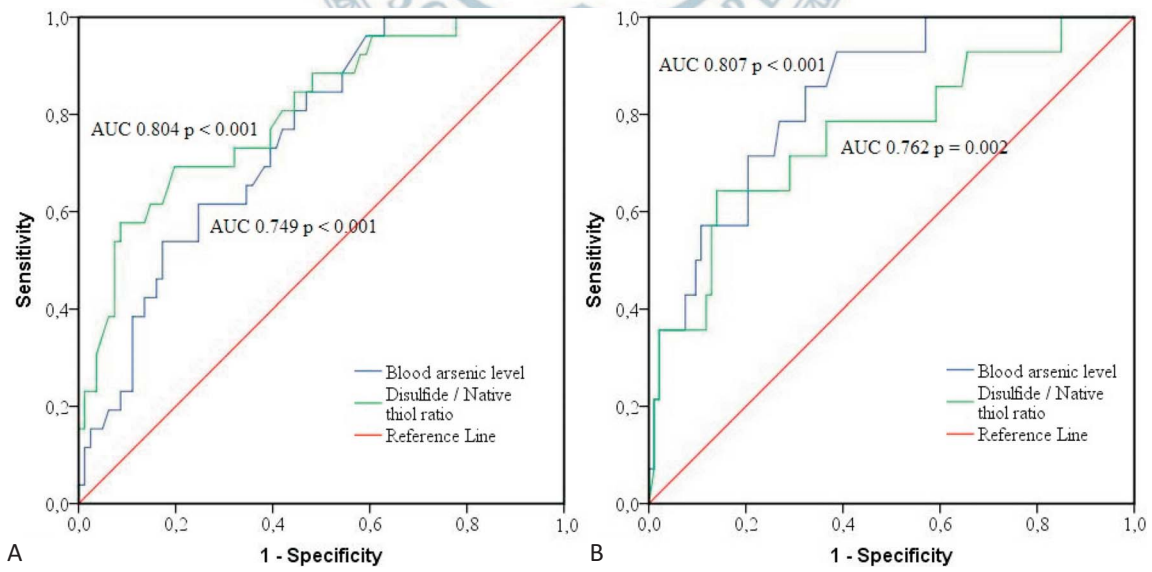

Figure 4. *Receiver operating characteristic curves. Blood arsenic level and disulphide/native thiol ratio for predicting increased LV diastolic pressure (A) and diastolic dysfunction (B) in the arsenic-exposed group.*

pensive, practical, fully automated, and also optionally manual spectrophotometric assay.[19]

In the present study, a strong association was found between arsenic exposure and LV diastolic dysfunction. The association between arsenic exposure and increased antioxidant status suggests that oxidative stress through thiol and disulphide homeostasis may play a role in the association between arsenic exposure and diastolic dysfunction. In the literature, the essential role of oxidative stress in heart failure, ischemic heart diseases, and myocardial and endocardial injuries has been emphasized.[27,28] Similar to the association between diastolic dysfunction and oxidative stress in the present study, in an experimental study with rats, Yang et al. demonstrated an increase in BP with chronic low-dose arsenic exposure, and argued that it was caused by arsenic-induced antioxidant suppression.[29] Furthermore, it has been suggested that antioxidant usage has the potential to improve the cardiovascular effects of arsenic exposure.[30] As a result, oxidative stress may be a common mechanism mediating the cardiovascular effects of arsenic (Figure 5).

Some studies have reported increases in total cholesterol and triglyceride levels[31] and decrease in high-density lipoprotein levels with arsenic exposure;[32] however, there was no difference in lipid profile in the arsenic-exposed group in this study. Similar to our study, Ledda et al. also found no significant differences in total cholesterol, triglyceride, low- and high-density lipoprotein concentrations in arsenic-exposed workers compared to controls.[33] On the other hand, both systolic and diastolic BP of the arsenic-exposed group were higher than that of the control group. This finding is consistent with previous studies which demonstrated the unfavorable effect of arsenic exposure on BP profile.[34,35] The effect of high BP on LV diastolic dysfunction has been reported; however, the association between average E/e′ ratio and BP was not found in correlation and regression analyses in the present study. This is likely to be due to the enrollment of normotensive subjects into this study. Our results indicate that arsenic has an unfavorable effect on diastolic function regardless of BP profile.

This study investigated associations among arsenic exposure, oxidative stress, and diastolic function. In cases of arsenic exposure, LV diastolic function may deteriorate without systolic dysfunction, and arsenic-exposed individuals should be thoroughly screened in terms of diastolic parameters and thiol-disulphide homeostasis to assess oxidative stress, which is one of the major mechanisms for arsenic-induced cardiac toxicity. The findings of this study may inspire researchers to perform further studies with regards to the prognostic and antioxidant-based therapeutic implications.

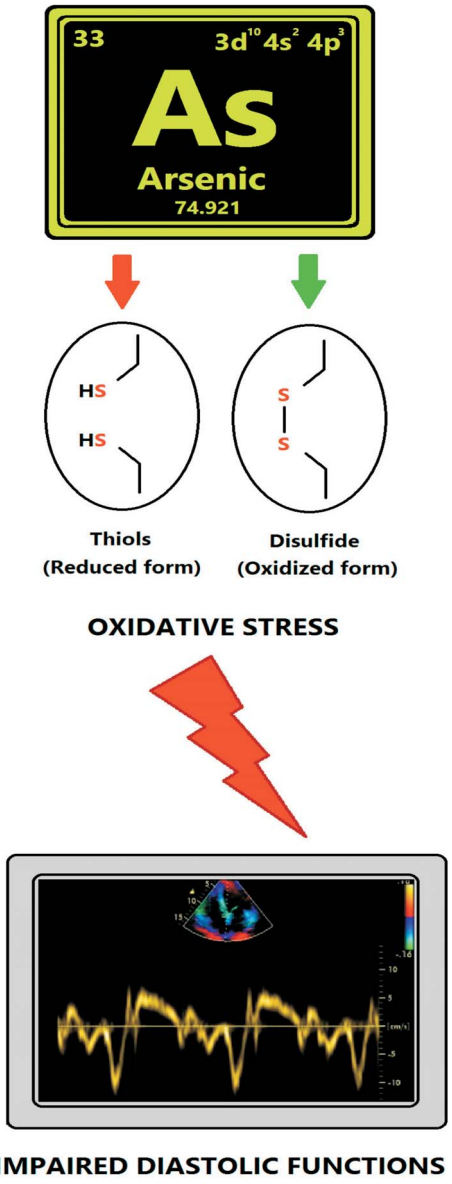

Figure 5. *The pathway from arsenic exposure to diastolic dysfunction. As a heavy metal, arsenic impairs oxidant and antioxidant homeostasis against thiols. Oxidative stress due to the decrease in thiols leads to myocardial damage, and this cellular mechanism results in diastolic dysfunction as a clinical manifestation.*

Study limitations

There are a number of limitations to this study. First, arsenic levels were studied only in blood. Arsenic accumulates in hair and nails due to its affinity for sulfhydryl groups.[36] Thus, arsenic levels in these samples are considered to be a better measure of long-term arsenic toxicity than blood level. Second, because of the cross-sectional design of this study, follow-up of the participants is not present, and cardiovascular end-points were not evaluated. Therefore, interpretation of the long-term cardiovascular effects of arsenic using the findings of this study is difficult. This study was primarily designed to investigate "exposed" populations, however the "the source of exposure" was not studied, and analysis of measurements of the work-place environment was not performed".

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CONFLICTS OF INTEREST

All the authors declare no conflict of interest.

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