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Urinary arsenic levels and risk of renal injury in a cross-sectional study in open population

María Ludivina Robles-Osorio,* Iván N. Pérez-Maldonado,‡ Daniel Martín del Campo,‡ Diana Montero-Perea,† Itzel Avilés-Romo,† Elizabeth Sabath-Silva,§ Ernesto Sabath*,‖

*Laboratorio de Endocrinología y Metabolismo, †Facultad de Medicina, Universidad Autónoma de Querétaro.
‡Laboratorio de Toxicología Ambiental, Facultad de Medicina, Universidad Autónoma de San Luis Potosí.
§Departamento de Fisiología y Biología Celular, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.
‖Servicio de Nefrología, Hospital General de Querétaro.

Niveles urinarios de arsénico y el riesgo de lesión renal en un estudio transversal en población abierta

RESUMEN

Introducción. *El arsénico (As) es uno de los metales pesados más abundantes en la naturaleza y la exposición prolongada a concentraciones elevadas de As se asocia con mayor riesgo de desarrollar cáncer, diabetes mellitus, hipertensión arterial y enfermedad cardiovascular. Hay pocos estudios acerca de la asociación de niveles de As y sus efectos en albuminuria, daño tubular y otras variables de laboratorio como el ácido úrico.* ***Objetivo.*** *Analizar la asociación entre la concentración urinaria de As con la albuminuria y excreción urinaria de α1-microglobulina como marcador temprano de daño tubular.* ***Material y métodos.*** *Estudio comparativo y transversal realizado en cinco comunidades cercanas a la ciudad de Querétaro (México). Los criterios de inclusión fueron personas sin antecedentes de enfermedad renal, hipertensión arterial, diabetes mellitus o exposición industrial al As. Se realizó un cuestionario acerca de factores de riesgo de exposición al As, mediciones antropométricas y de presión arterial, se tomó una muestra de sangre venosa, así como una muestra de orina al azar para el análisis de albúmina, α1-microglobulina y As.* ***Resultados.*** *Se incluyeron 90 personas sin antecedentes de enfermedad renal, diabetes o hipertensión arterial; la edad promedio de la población fue de 40.9 ± 12.9 años de edad y la mediana de la concentración urinaria de As fue de 15 μg/gr Cr (rango 0.56-89.2 μg/gr Cr), diez personas tuvieron niveles críticos > 50 μg/grCr. La edad mayor de 50 años [OR 2.48 IC95 (0.9-6.6)] y el lugar de residencia fueron los factores de riesgo más importantes asociados a concentraciones más elevadas de As. La excreción urinaria de As se encontró asociada a mayor excreción de α1-microglobulina (r^2 = 0.07) como marcador de daño tubular temprano, no encontramos asociación con albuminuria u*

ABSTRACT

Introduction. Arsenic (As) is one of the most ubiquitous elements in nature, and a prolonged exposure has been associated with an increase in the risk of cancer, diabetes mellitus, hypertension and cardiovascular disease. There are few studies addressing the effects of As on albuminuria, tubular injury and biochemical variables as uric acid. **Aim.** To analyze the association between urinary As levels, albuminuria, and α1-microglobulin as marker of tubular injury. **Material and methods.** This is a cross-sectional, and comparative study done in 5 communities localized close to Queretaro City. Subjects with no antecedents of renal disease, diabetes, hypertension, or industrial exposure to As were included. A questionnaire about risk factors for arsenic exposure was done, blood was taken for biochemical analysis and a spot urine sample was collected for albumin, α1-microglobulin, and As measurements. **Results.** A total of 90 adult persons were included with no antecedents of renal disease, diabetes or hypertension; the mean age was 40.9 ± 12.9 years and the median for urinary As levels was 15 μg/gr Cr (range 0.56-89.2 μg/gr Cr), 10 (11.1%) persons had critical levels > 50 μg/gr Cr. Age more than 50 years old [OR 2.48 IC95 (0.9-6.6)] and place of residence were the most important risk factors associated with higher levels of As. There was association between urinary As levels and α1-microglobulin urinary excretion (r^2 = 0.07, p = 0.01) but not with albuminuria or other biochemical variables. **Conclusions.** This is the first study in Mexico to show an association between As and urinary excretion of α1-microglobulin as marker of early renal injury. We did not found association with albuminuria or other serum biochemical variables. Arsenic may be considered as a risk factor for tubular injury.

otros marcadores bioquímicos en suero. ***Conclusiones.*** *Éste es el primer estudio en México en demostrar asociación entre los niveles de As y la excreción urinaria de α1-microglobulina como marcador de daño renal temprano. No encontramos asociación con albuminuria u otras variables bioquímicas. El As debe ser considerado como un factor de riesgo para el desarrollo de lesión renal temprana.*

Palabras clave. *Albuminuria. α1-microglobulina. Arsénico. Insuficiencia renal crónica.*

Key words. Albuminuria. α1-microglobulin. Arsenic. Chronic kidney disease.

INTRODUCTION

Arsenic is one of the most common heavy metals in nature; people are exposed through environmental, occupational, and rarely by medical sources (some treatments for leukemia and leishmaniasis contain As).[1] In Mexico, exposure occurs mainly by drinking contaminated water and there are some areas, as La Laguna (Coahuila), that As contamination is cause of great concern. In Queretaro City, drinking water comes from groundwater and recently from Zimapan, which has one of the highest As concentrations in Mexico.[2] Concentrations of urinary As levels < 10 μg/gr Cr have no effects on health, but values > 50 μg/gr Cr are critical and poses a considerable risk for adverse health effects.

Chronic exposure usually is asymptomatic and health-related problems may manifest many years after the exposure occurred; however, some patients may present with skin lesions, peripheral neuropathy and anemia.[3] Chronic exposure to even low levels is associated with a higher risk to develop cancer,[2] diabetes mellitus,[4]arterial hypertension,[5] and atheroesclerosis.[6] In children, As affects mostly central nervous system with attention disorders.[7]

There are few reports about the effects of As on renal function, and animal studies have shown that As induce renal injury with decrease in glomerular filtration rate (GFR), low-weight proteinuria (LWP), albuminuria and increase in uric acid levels.[8] In humans, Meliker, *et al.*,[9] in one study done in Michigan (USA) reported a higher mortality related to renal diseases in areas with higher arsenic concentration. Hsueh, *et al.*,[10] in Taiwan showed an association ($r^2 = 0.04$) between GFR and urinary As; however, limited information is available on the association of low exposure to As with albuminuria and other markers or renal injury.

In Mexico, this is the first report about urinary As levels and its association with albuminuria and α1-microglobulin excretion as marker of tubular injury and early renal dysfunction.

OBJECTIVE

The aim of this study was to know urinary As levels and its relationship with albuminuria, α1-microglobulin and other biochemical variables in healthy population without industrial or mining exposure to As.

MATERIAL AND METHODS

This is a cross-sectional and comparative study approved by the Bioethics Committee of the University of Queretaro Medical School (FM-UAQ) and informed consent was obtained of each participant. The study was done in 5 communities (Santa Rosa Jaúregui, San José el Alto, Miranda, Santa Bárbara, and Lomas de Casablanca) (Table 1) located close to Queretaro City (Central Mexico) from March to April of 2010, and a probabilistic sampling procedure was used to recruit the population.

We included subjects with no antecedents of renal disease, diabetes, hypertension, industrial exposure to As, and aged > 18 years old. Pregnant women and people with current urinary tract infection were excluded.

A questionnaire was used to obtain information on demographic and socioeconomic aspects, family health history, personal medical history, and risk factors to As exposure. Anthropometric and blood pressure measurements were obtained after a 5 min

Table 1. Urinary arsenic concentrations in adult population of 5 communities in Queretaro (Central Mexico).

Community (n)	Median As concentration (μg/gr Cr)	% As > 50 μg/gr Cr (n)
Lomas de Casablanca (5)	8.47	0 (0)
Miranda (16)	2.30	6.25 (1)
San José el Alto (20)	15.3	10 (2)
Santa Bárbara (22)	13.0	13.6 (3)
Santa Rosa Jáuregui (27)	21.3	14.8 (4)

resting. During the same visit and after overnight fasting, blood and first urine samples were obtained and the analytical measurements were done at the core laboratory of the FM-UAQ. Arsenic measurements were done at the Department of Environmental Toxicology Laboratory (San Luis Potosi Medical School). Glucose, urea, creatinine and uric acid measurements were done by standard technique. GFR was calculated with the Modification Diet and Renal Disease (MDRD) formula.

Spot urine samples for albumin, α1-microglobulin and arsenic analysis were collected in arsenic-free containers, and stored frozen at -80 °C until their analysis. Albumin, α1-microglobulin, and As were creatinine adjusted. The α1-microglobulin determinations were done with the Alpco Immunoassays Kit ELISA[33] according to the manufacturer instructions. Albuminuria was considered abnormal with a ratio > 0.3 g/g and the α1-microglobulin with a ratio > 10 μg/g.

Urine samples were wet-digested for As quantification. The quantification of As was carried out with a Perkin-Elmer 3110 atomic absorption spectrophotometer and then assayed by the hydride-evolution technique. For quality control an Iris Clinchek control test (lyophilized urine control level I and II; GmbH Labortechnik D80335 Munich/Germany) was used with an accuracy of 98%.

Statistical analysis

For descriptive statistics mean ± standard deviation, or percentages as appropriate were calculated. Comparisons between groups for variables with normal distribution were done by t-student analysis, and for those with non-normal distribution non-parametric statistics (Fisher test) was done. Multivariate analysis was performed to identify risk factors for high As levels. Linear correlation analysis with Pearson and coefficient of determination was used to determine correlation between continuous variables. For variables correlated with α1-microglobulin concentration multiple linear regression analysis was used. A p value < 0.05 was considered as statistically significant. The data were analyzed using the SPSS 16.0 program.

RESULTS

A total of 90 persons were included; the mean age was 40.9 ± 12.9 yrs and most of participants were females (77.8%), and the general characteristics of all participants are shown in table 2. The median for

Table 2. Demographic, anthropometric and laboratory characteristics of the study sample.

	n = 90
Age (years)	40.9 ± 12.9
Female (%)	77.8%
Smoking (%)	16.6%
SystolicBP (mmHg)	114.9 ± 16.3
DyastolicBP (mmHg)	75.4 ± 7.6
BMI (body mass index)	27.5 ± 4.9
GFR (ml/min)	83.4 ±16.5
GFR< 60 ml/min (%)	12.2
α1-microglobuline > 10 (%)	8.8
Albuminuria (%)	4.4
Glucose (mg/dL)	92.5 ± 9.4
Urea (mg/dL)	27.9 ± 7.3
Creatinine (mg/dL)	0.85 ± 0.17
Uric acid (mg/dL)	4.4 ± 1.4

Table 3. Associated risk factors for As concentrations > 15 μg/gr Cr.

Risk Factor	OR (IC 95%)	p
• Age		
< 50 years old	1	
> 50 years old	2.48 (0.9-6.6)	0.05
• Smoking		
No	1	0.2
Yes	1.75 (0.56-5.61)	
• Gender		
Female	1	0.4
Male	0.79 (0.28-2.17)	
• Place of residency		
Lomas de Casablanca (Queretaro Downtown)	1	0.0003
Santa Rosa Jáuregui (Suburban Community)	13.2 (2.97-58)	

urinary As levels were of 15.0 μg/gr Cr (range 0.56-89.2 μg/Cr), and 10 (11.1%) persons had critical levels > 50 μg/gr Cr.

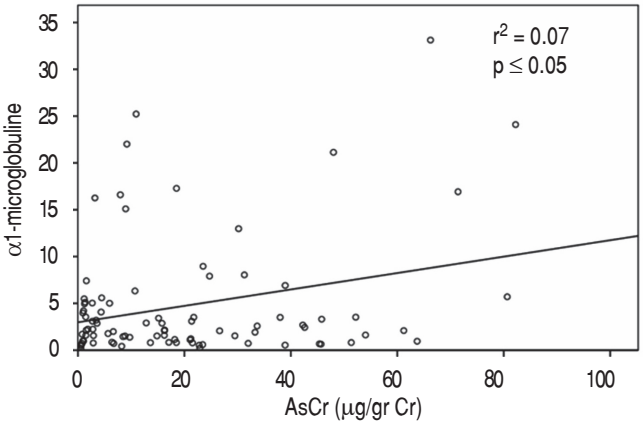

Figure 1. Correlation between the urinary excretion of α1-microglobulin with urinary arsenic excretion.

Table 4. Linear regression analysis of different variables associated with higher urinary excretion of α1-microglobuline.

Variable	Standardized coefficients beta	p
Arsenic	-0.109	0.001
GFR	-0.307	0.014
Age	-0.109	0.343
BMI	0.101	0.349
SBP	-0.046	0.704
Glucose	0.098	0.368
Uric acid	0.147	0.188

Dependent Variable: α1-microglobuline/creatinine ratio. GFR: glomerular filtration rate. BMI: body mass index. SBP: systolic blood pressure.

Conditions associated with an increase likelihood of higher levels of urinary As were age > 50 years old [OR 2.48 IC95 (0.9-6.6) p = 0.05], and place of residency [OR13.2 IC95 (2.97-58) p 0.003] being Santa Rosa Jaúregui the community with higher As levels (Table 3).

We performed a correlation analysis of urinary As levels with albuminuria, α1-microglobulin, and other laboratory variables and we found that arsenic was significantly associated with α1-microglobulin urinary excretion (Figure 1); in multivariate analysis As was the only factor associated with an increased urinary α1-microglobulin excretion (Table 4). There was no correlation of As concentration with albuminuria, and other biochemical variables.

We also compare Q1 *vs.* Q4 (Table 5) arsenic levels for α1-microglobulin excretion and those patients in percentile Q4 had higher α1-microglobulin urinary excretion (3.2 ± 3.4 *vs.* 8.8 ± 6.2 μg/gr Cr p = 0.05). The α1-microglobulin levels were not affected by anthropometric measurements, albuminuria or blood pressure. Those patients in percentile Q4 also had significantly higher uric acid levels (3.8 ± 1.2 *vs.* 4.5 ± 1.5 mg/dL p = 0.03), however none of the participants refers a history of gout. Interestingly those patients with an abnormal α1-microglobulin excretion had significantly higher As concentration (19.2 ± 19.8 *vs.* 30.2 ± 28.9 μg/gr Cr p = 0.04).

By the characteristics of the population selected we did not expect to find many patients with chronic kidney disease (CKD); however we found 11 (12.2%) persons with GFR < 60 mL/min. Comparing with individuals with normal GFR, those with GFR < 60 mL/min were older (49 ± 7 *vs.* 39.9 ± 13 years p = 0.01), and had higher levels of systolic

Table 5. Comparison of biochemical variables between arsenic levels percentile 25 and 75.

	Q1 (n = 23)	Q4 (n = 23)	p
Age (years)	39.8 ± 9.6	43.0 ± 12.3	NS
Female (%)	91.4	82.7	NS
Smoking (%)	4.3	17.3	0.05
Systolic BP (mmHg)	112.3 ± 15.2	118.4 ± 22.6	NS
Dyastolic BP (mmHg)	75.2 ± 7.3	75.4 ± 8.2	NS
BMI (body mass index)	27.0 ± 4.7	27.0 ± 4.2	NS
GFR < 60 mL/min (%)	17.3	13.0	NS
α1-microglobuline > 10 μg/gr Cr (%)	4.3	17.3	0.05
Albuminuria (%)	4.3	4.3	NS
Glucose (mg/dL)	92.0 ± 8.6	92.6 ± 8.3	NS
Urea (mg/dL)	29.0 ± 7.0	27.3 ± 7.9	NS
Creatinine (mg/dL)	0.88 ± 0.1	0.81 ± 0.1	NS
Uric acid (mg/dL)	3.8 ± 1.2	4.5 ± 1.4	0.03

blood pressure (121 ± 10 vs. 113.7 ± 12.7 mmHg p = 0.007); however, the median As concentration was not different (13.6 *vs.* 16.2 μg/gr Cr p = NS) between the two groups.

DISCUSSION

This is the first report in Mexico about the effects of As and its correlation with early renal injury in persons with non-industrial exposure to As, also this is the first report of renal tubular injury in healthy Mexican population.

The glycoprotein α1-microglobulin has a low molecular weight (26-31 kDA), is filtered through the glomeruli and completely reabsorbed in proximal tubule. Injury to tubular cells increases the urinary levels of this protein. This protein has been extensively studied as a marker of tubular injury in many diseases as heavy metals nephropathy, hypertension, diabetes mellitus and multiple myeloma so we do not consider that the results of this study change using other markers of tubular injury as NGAL or KIM-1, etc.[11-13] Some studies have found that α1-microglobulin increases in very early stages of CKD even before creatinine or GFR decline.[14,15]

The effects of As on kidney are complex and not completely studied, although some studies done in animals have shown that mice with exposure to As develop glomerular sclerosis and tubular necrosis.[16] Arsenic is absorbed in intestine, lung and skin and is widely distributed in the human body. Arsenics is filtered through the glomeruli and reabsorbed in the proximal segments of the nephron; As toxicity is related to depletion of the glutathione stores and increase in free radicals.[17-19] Urinary arsenic is a measure of total body burden.

The percentage of adult population with As concentration in critical level above 50 μg/gr Cr was higher than expected, and more studies are necessary to detect the potential source of As in Queretaro; however, is likely that this problem is related to water because in Queretaro most of water used to drink is obtained from groundwater and recently from Zimapan which has one of the highest As concentrations in the world. In a previous study done in children in Queretaro, Trejo-Acevedo, *et al.* found a mean urinary As concentration of 14.1 μg/gr Cr.[20]

High urinary As levels are associated with other health problems as hypertension, atherosclerosis, diabetes mellitus and carcinogenesis so the finding that persons with non-industrial exposure to As had higher levels than expected may be a risk factor to develop this morbidities.[1,2]

Another finding of this study was the high prevalence of individuals with GFR levels below 60 mL/min; this finding highlights the importance to search for other risk factors involved in the decrease of GFR of this population. Also, is important to extend this study and include persons with chronic kidney disease to have a better idea of the effects of As on kidney function in our population. Other limitation of this study is the small sample size so the statistical significance should be interpreted with caution.

In conclusion, this study shows that urinary As levels are significantly correlated with α1-microglobulin urinary excretion, and that in our population age and place of residency were the most important risk factors for higher urinary As concentrations. Further studies are warranted to dilucidate the role of arsenic in renal disease in Mexican population.

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Correspondence and reprint request:

Dr. Ernesto Sabath
Unidad Estatal de Hemodiálisis
Fray Luis de León, Núm. 2990
Col. Centro Sur
76090, Querétaro. Qro.
Tel.: 52 442 2 29 1778
Correo electrónico: esabath@yahoo.com

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