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Lung inflammation biomarkers and lung function in children chronically exposed to arsenic

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Abstract

Evidence suggests that exposure to arsenic in drinking water during early childhood or *in utero* is associated with an increase in respiratory symptoms and diseases in adulthood, however only a few studies have been carried out during those sensitive windows of exposure. Recently our group demonstrated that exposure to arsenic during early childhood or *in utero* was associated with impairment in the lung function in children and suggested that this adverse effect could be due to a chronic inflammatory response to the metalloid. Therefore, a cross-sectional study was designed in a cohort of children associating lung inflammatory biomarkers and lung function with urinary As levels. A total of 275 healthy children were partitioned into four study groups according with their As levels. Inflammation biomarkers were measured in sputum by ELISA and the lung function was evaluated by spirometry. Fifty eight percent of the studied children were found to have a restrictive spirometric pattern. In the two highest exposed groups, the Soluble Receptor for Advanced Glycation Endproducts (sRAGE) sputum level was significantly lower and Matrix

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Metalloproteinase-9 (MMP-9) concentration was higher. When the biomarkers were correlated to the urinary arsenic species, negative associations were found between dimethylarsinic (DMA), monomethylarsenic percentage (%MMA) and dimethylarsinic percentage (%DMA) with sRAGE and positive associations between %DMA with MMP-9 and with the MMP-9/Tissue Inhibitor of Metalloproteinase (TIMP-1) ratio. In conclusion, chronic arsenic exposure of children negatively correlates with sRAGE, and positively correlated with MMP-9 and MMP-9/TIMP-1 levels, and increases the frequency of an abnormal spirometric pattern.

Keywords

Soluble Receptor for Advanced Glycation Endproducts (sRAGE); Metalloproteinase-9 (MMP-9); Tissue Inhibitor of Metalloproteinase (TIMP-1); Lung inflammation; Sputum; Arsenic

Introduction

Chronic arsenic (As) exposure through drinking water has been correlated with increased incidence and mortality by cancers as well as nonmalignant diseases (Chiou et al, 1995; Hopenhayn-Rich et al, 1998). While arsenic can affect many tissues and organ systems, the lung seems particularly susceptible (NRC, 2001). Although the mechanism of As-induced respiratory illness is not fully understood, several studies have shown that a large amount of As is deposited and stored in the lung, especially in the epithelium (Gerhardsson et al, 1988; Saady et al., 1989). In rabbits, arsenic has been shown to accumulate in the lung more than in any other organ except the liver and kidney (Bertolero et al., 1981; Marafante et al., 1981).

Several studies have reported a high prevalence of respiratory symptoms in subjects highly exposed to As through drinking water such as chronic cough, abnormal chest sound, shortness of breath (Mazumder et al., 2000), lower forced expiratory volume measured in 1 second (FEV₁), and altered forced vital capacity (FVC) (von Ehrenstein et al., 2005). Additionally, De et al. (2004), reported that 57% of subjects chronically exposed to arsenic had respiratory symptoms with 53% having restrictive lung disease and 41% of the study participants having both obstructive and restrictive lung diseases. Mazumder et al. (2005), reported a 10-fold increased risk of chronic obstructive pulmonary disease, identified by high-resolution computed tomography in subjects chronically exposed to arsenic. In a study carried out by Smith et al. (2006), they found that exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults from both malignant and nonmalignant lung disease. Recently our research group demonstrated that exposure to arsenic through drinking water during *in utero* and early life was associated with a decrease in FVC and with a restrictive spirometric pattern in the children that suggested that these adverse effects could be due to a chronic inflammatory response to this metalloid (Recio-Vega et al., 2015). Therefore, it is very important to carry out studies with the aim of detecting early lung diseases in order to decrease the frequency of pulmonary pathologies during the adulthood.

Several authors have suggested that the mechanism of action of As in the lungs is that it can enhance tissue inflammation (De et al., 2004; Nemery, 1990), induce respiratory function

impairment by oxidative stress (Lantz RC & Hays AM, 2006) and/or produce or increase pulmonary fibrosis (von Ehrenstein et al, 2005; Nemery, 1990). Increased inflammatory responses have been reported in infants born to arsenic exposed mothers (Fry et al., 2007) and arsenic alters markers of inflammation (sRAGE, MMP-9 and TIMP-1) in adults exposed to 20 µg/L As. sRAGE has been recognized for its role in several chronic diseases, such as diabetes, atherosclerosis, coronary artery disease, and lung cancer (Bierhaus et al., 2005; Falcone et al., 2005; Hofmann et al., 2004). In a population-based study, Lantz et al. (2007), demonstrated a significant negative correlation between sRAGE sputum levels and total urinary inorganic As. MMPs and TIMP-1 are sensitive markers of lung inflammation in humans (Josyula et al., 2006) and both of them are continually secreted in the airways. *In vitro* models have shown that acute arsenic exposure increases activity and expression of MMP-9 in airway epithelial cells (Olsen et al., 2008). Josyula et al. (2006) evaluated the changes in biomarkers of lung inflammation, as measured by the ratio of sputum metalloproteinase and antiprotease activity, in subjects exposed to arsenic and concluded that the increased sputum proteinase/antiprotease activity suggests a potential toxic mechanism for low-level arsenic exposure. Human and mouse models have shown that *in utero* and early life exposures to arsenic can result in alterations in adult lung function and lung disease. However, no reports exist concerning the relationship of long-term exposure to As with sRAGE, MMP-9 and TIMP-1 sputum levels or between these inflammation biomarkers and lung function in children. Studies at this age will provide insight of developing lung injury and allowing for initiation of preventive programs with the aim of reducing pulmonary diseases in children and later in adulthood. In this study Mexican children from a rural area with arsenic present in their drinking water were examined for urinary arsenic levels, spirometric lung function, and inflammatory markers in their sputum.

Material and methods

Study population

The subjects included in this report are a subset of those reported in an earlier study (Recio-Vega et al., 2015). More than 500 children were evaluated; however since satisfactory sputum sample is not easy to obtain at this group-age, we only include in this study those children where acceptable sputum samples were obtained (n=275) allowing us to better assess the risk to children from arsenic exposure. The participants were females and males aged 6-12 years residing in four rural communities in which the highest arsenic tap water levels have been detected in the last 20 years (104-360 ppb) in the studied area. These communities received groundwater through the local water supply and the high As levels detected are due to an over water extraction from the ground for crops. At present, water is obtained from a depth of 200-300 meters. These communities form part of the geographic area known as Comarca Lagunera, which is located in the north-central part of Mexico. To aid in focusing on long-term and continuous arsenic exposure we only included subjects, who were conceived in the studied rural communities, whose mothers lived their entire pregnancies in these communities, and who have remained as permanent residents of the same communities. Subjects who agreed to participate in the study were divided in four study groups (quartiles) according with their total urinary arsenic level (Group 1: 63 µg/L; Group 2: 63-113 µg/L; Group 3: 114 - 181 µg/L; and Group 4: >181µg/L).

Written informed consent was obtained from each participant and from their parents to obtain biological samples. The study protocol was approved by the Ethics Committee of the School of Medicine at Torreon, University of Coahuila, Mexico.

Questionnaire application

Information was collected through in-person interviews and included socio-demographic variables (education, socioeconomic status, type of kitchen, type of fuel used for cooking), lifetime residential history, lifestyle factors (secondhand smoke defined as someone smoking regularly in the same room at home, and exercise), parent's occupational history, water source types (municipal tap water, bottled, other), current medications, medical history, and diet. Water consumption habits were ascertained through a standardized questionnaire. Standardized questions were adapted to Spanish from the questionnaire used by the American Thoracic Society Division of Lung Disease (Ferris, 1978) to register the presence of respiratory symptoms. Asthmatic volunteers confirmed by a doctor were not included in the study. Written informed consent was obtained from each participant and from their parents to obtain biological samples, which were obtained at the time of interview.

As measurement in drinking water and urine

Three drinking water samples (school, home and well) were collected from each rural community included in the study and analyzed for inorganic arsenic levels. No other contaminants in the drinking water were assessed. Individual exposure was assessed based on urinary concentration of the sum of inorganic As. A first morning void urine sample was collected in sterile 120-mL screw-topped polypropylene container. The urine samples were obtained during the late autumn and winter seasons to avoid the hottest seasons when there is much higher water consumption and children have increased outdoor activities. Urine samples were analyzed using the methodology described by U.S. Center for Disease Control (CDC, 2004) at the Arizona Laboratory for Emerging Contaminants, University of Arizona, Tucson, Arizona, U.S.A. Briefly, arsenic species in urine (As^{V} , As^{III} , monomethylarsonic acid (MMA^{V}), dimethylarsinic acid, (DMA^{V}) and arsenobetaine) were separated by HPLC. Arsenic concentrations in water samples and urine were analyzed by inductively coupled plasma mass spectrometry utilizing the Standard Reference Water, SMR 1640 (NIST, Gaithersburg, MD, USA) and the freeze-dried Urine Reference Material for trace elements (Clinchek-control; RECIPE Chemicals instruments GmbH, Munich, Germany) for urine as quality control. Urinary arsenic concentration was adjusted for creatinine by expressing its level as a ratio with creatinine ($\mu\text{g/g}$ creatinine). The creatinine levels were estimated in urine samples using the Jaffe reaction method. Additional exposure to DMA or to organic arsenic metabolized in the body to DMA, which usually is attributable to consumption of seafood such as bivalves and seaweeds, was considered minimal because such sea-foods are essentially never eaten in this area. Arsenic metabolism efficiency was calculated using the following formulas proposed by Del Razo et al. (1997), first methylation = $\text{MMA}^{\text{V}}/(\text{As}^{\text{V}} + \text{As}^{\text{III}})$; second methylation = $\text{DMA}^{\text{V}}/\text{MMA}^{\text{V}}$.

Lung function measurement

A detailed description of lung function measurements on children was reported in our previous study (Recio-Vega et al., 2015). Briefly, lung function was assessed according to American Thoracic Society guidelines (1995) using an EasyOne spirometer (NDD Medical Technologies, Zurich, Switzerland). The device was standardized to meet American Thoracic Society guidelines for lung function tests. Predicted values were those of the Mexican-Americans in NHANES III (Hankinson et al., 1999). Each subject's best trial (largest sum of FEV₁ and FVC) was included in analyses. The severity of pulmonary restriction was considered using the % of predicted FVC registered (low 70 to lower normal value; moderate 60 <70; severe 34 <50). Spirometry data were reviewed by a pulmonologist.

Sputum analysis

Sputum induction was performed by using a sterile 5% saline aerosol (Baxter, Deerfield, IL, USA) generated by using Comp Air XLT, Compressor Nebulizer Systems (Model NE-C25, Omron Healthcare, Bannockburn, Illinois, USA) set on maximum output. Subjects were encouraged to cough up every 2 min for a period of 30 min and the sputum was collected. Prior to each cough, subjects were asked to discard any saliva to minimize salivary contamination of the sputum sample. Sputum samples were treated with 10% sputolysin (Calbiochem, San Diego, CA, USA) containing penicillin streptomycin to inhibit bacterial growth. Samples were then centrifuged to separate cells from the supernatant, which was stored at -70 °C (Burgess et al., 2013). Sputum supernatant samples were analyzed in duplicate for levels of sRAGE, MMP-9, and TIMP-1 using a commercially available enzyme-linked immunosorbent assay (ELISA) (Biovendor LLC, Asheville NC 28806 USA). A uniform initial sample dilution was performed on all samples to maximize the number of samples with concentrations within the standard range for the sRAGE, MMP-9, TIMP-1 ELISA. Total protein concentration was estimated using the Lowry's method. The detection limits for sRAGE, MMP-9, TIMP were 50 pg/mL, 0.23 pg/mL, and 0.39 pg/mL, respectively.

Statistics

Independent and dependent variables were described according to their frequency and distribution measurements (arithmetic mean and standard deviation). The F test was used when the variable was divided into more than two categories, and the Student's t test or the Mann Whitney test was used to compare different levels of arsenic when dichotomic variable categories were analyzed. This method permitted us to establish statistical differences among groups for each dependent variable. For statistical analysis and because protease and antiprotease values did not show a normal distribution, their levels were converted to logarithm and thereafter re-transformed to normal values. Linear regression models were used to assess crude or independent associations between subjects' variables with arsenic urine concentrations. Linear regression analysis was also performed separately for FEV₁, FVC and FEV₁/FVC ratio as well as, for sRAGE, MMP-9, TIMP-1 and MMP-9/TIMP-1 in order to determine the association of percentage predicted lung volumes with respiratory symptoms and with the arsenic levels. Validity of the ATS respiratory

questionnaire along with predictive values was also calculated for obstructive and restrictive lung patterns separately. In multiple analyses, all relevant variables identified in the bivariate model were included in addition to those possessing biological plausibility. Crude and adjusted Odds Ratios was calculated too. All analyses were performed using the statistical software STATA 11.0 (Stata Corp., College Station, TX).

Results

Sociodemographic data

Most of the socio-demographic, anthropometric characteristics, and life style variables of the volunteers were similar between groups of study, except, age, BMI and schooling which were lower in subjects from Group 4 ($p<0.05$), whereas, months of breastfeeding was lower in children from Group 3 ($p<0.05$) (Table 1). The frequency of use of tap water for cooking was slightly higher in subjects from Group 4, however, it was not significantly different when compared with the other groups ($p=0.08$). No differences were found in the number of glasses of tap water drank per day, either at home or at school, between the studied groups ($p>0.05$) (Table 1).

Urinary and drinking water As levels

The mean As water concentration at time of this study in the four communities evaluated was 103.2 $\mu\text{g/L}$ (range: 42.9–181.4) (Table 2). The total urinary As, iAs, and methylated metabolites correlated to the increased mean arsenic water concentration across the groups.

Although substantial increases in the amount of monomethylated (MMA) metabolites were seen in the higher exposed groups, the % monomethylated metabolite was about the same. In contrast, there was a large increase in the % dimethylated metabolites in the higher exposed groups. This range of arsenic exposures and the span of arsenic concentrations in the urine of the subjects provide a good foundation to determine if the pulmonary inflammatory markers and the lung function test are related to arsenic exposure.

Respiratory symptoms, lung function and inflammatory biomarkers

Symptoms: The frequency of chronic phlegm was significantly higher in subjects from Group 3 than that recorded in Group 1 ($p<0.05$) (data not showed). The respiratory symptoms most common registered in the studied population were cough and chronic cough, which were slightly more frequent in subjects from group 3, 4 and 2, but not significantly more than group 1 ($p>0.05$).

Lung Function: When the lung function was evaluated in the children studied, a slight tendency ($p>0.05$) toward decreased lung function was observed in accordance with exposure level (Table 3). When the results of FEV₁ and FVC were re-analyzed with the aim of obtaining a spirometric pattern, the restrictive pattern was registered in a total of 159 subjects (57.8%). From the children with this restrictive pattern, 96.8% of them had a low severity grade, 2.5% moderate severity grade, and only 1 subject (0.62%) had severe grade of restrictive lung disease. The higher frequency of abnormal spirometry pattern was observed in the most exposed groups (Table 3).

Inflammatory Biomarkers: With regards to the lung inflammatory biomarkers evaluated, the results obtained showed that the sRAGE sputum levels were significantly lower in subjects from Groups 2 and 3 compared to the children from Group 1 ($p<0.05$), meanwhile, the MMP-9 sputum concentration was significantly higher in subjects from Group 2 when compared with the levels found in Group 1 ($p<0.05$) (Table 4).

Correlations between As levels and lung function

In children with a diagnosis of restrictive spirometric pattern, DMA^v, first methylation, second methylation and %DMA levels were significantly higher ($p<0.05$) than in those subjects with normal spirometric pattern (Table 5). When the total arsenic urinary levels were compared in volunteers according to severity of the restrictive pattern, these levels were 2.3 times higher ($p=0.01$) in subjects with moderate restrictive pattern (336.4 µg/g) than the levels registered in children with low restrictive pattern (146.48 µg/g). When the association between abnormal lung function and arsenic urinary levels was evaluated, a negative relationship was found with the second methylation (β 0.027; 95%CI 0.007–0.04; $p=0.006$) and with %DMA^v levels (β 0.002; 95%CI 0.0002–0.004; $p=0.002$); these associations persisted after the adjustment of the statistical model with several covariables. Similarly, when the Odds Ratios was calculated (crude and adjusted) for abnormal spirometric pattern, an increased risk was found with the second methylation and %DMA levels (Table 6).

Correlations between biomarkers and lung function

When the inflammatory biomarkers were assessed according to the spirometric pattern, sRAGE and the MMP-9/TIMP-1 levels were significantly lower in subjects with the restrictive pattern (Table 5). In subjects with the diagnosis of moderate restrictive pattern, the MMP-9/TIMP-1 levels (0.48 ± 0.42 ng/mL) were lower ($p<0.05$) when compared with the found in volunteers with low restrictive pattern (7.6 ± 16.52 ng/mL) and with normal spirometric pattern (18.2 ± 60.3 ng/mL). When the risk for abnormal spirometric pattern was evaluated, a decrease in the risk was found with sRAGE and MMP-9/TIMP-1 levels (Table 6).

Correlations between biomarkers and As

In the bivariate analysis, sRAGE was negative and significantly associated with DMA^v, first and second methylation, %MMA^v and with %DMA^v ($p=0.02$). MMP-9 was positively associated with DMA^v, first and second methylation and with %DMA^v ($p<0.02$). The MMP-9/TIMP-1 levels were positively associated with DMA^v, second methylation and with %DMA^v ($p<0.01$). When the statistical model was adjusted by different co-variables, sRAGE maintained its association with DMA^v, second methylation, %MMA^v and with %DMA^v; meanwhile, MMP-9 was associated with the second methylation and with %DMA^v and the MMP-9/TIMP-1 levels with the second methylation and with %DMA^v (Table 7).

Discussion

To our knowledge, this is the first study evaluating the association between arsenic exposure and sputum inflammatory biomarkers in children chronically exposed to this metalloid. Previous studies have shown similar alterations in adults exposed to low levels of arsenic. This is the first to show that similar alterations in these arsenic-induced inflammatory biomarkers were shown to occur early in life and are associated with a decrement in lung function.

Arsenic levels and lung function

The participants studied were exposed since pregnancy until early childhood to an average of As water concentration of 152.1 ± 49.4 µg/L (range 104-275 µg/L). The mean urinary As level registered in the overall studied subjects was 148.2 µg/g. From all children evaluated, only 15.6% had a urinary concentration below of 50 µg/g.

In our work, most of the arsenic metabolites were more than 7 times higher ($p < 0.05$) in the two most exposed groups when compared with Group 1 ($p = 0.000$). For our questionnaire, the main source of the As levels in the children studied is ingestion and use of tap water; other As sources such as diet, environmental pollution or other compounds containing arsenicals (agrochemicals, preservatives, fuels) were negligible.

Evidence suggests that arsenic exposure in drinking water has been associated with an increase in respiratory symptoms or diseases in exposed adults. Sensitive windows of exposure may exist during development that can affect adult function. During pregnancy *in utero* transplacental transfer of arsenic occurs in humans (Concha et al., 1998) which can lead to functional changes via altered fetal programming (Heindel JJ., 2007; Vahter M., 2008). *In utero* exposure to arsenic reduces birth weight, and increases susceptibility to lower respiratory tract infections in infancy (McCall and Acheson, 1968), to impaired lung function during childhood (Chan et al., 1989), and to increased risk of death from chronic lung diseases in adulthood (Barker et al., 1991). Taken together these data suggest that arsenic induces impairments in growth and development of the lung (Ramsey et al., 2013). In our study, we did not find any significant differences in the prevalence of respiratory symptoms or diseases between groups, except, chronic phlegm which was significantly higher in subjects from Group 3 than recorded in the least exposed group ($p < 0.05$). The lower incidence of respiratory symptoms found in our study and in contrast with other studies, may be due to the level of As exposure and/or to the difference in age-group studied. The respiratory symptoms and diseases were not associated with As urinary levels or its metabolites in our study. These data are in agreement with those reported by Dauphine et al. (2011), who found no evidence of associations between arsenic levels and chronic cough, phlegm and chronic bronchitis in adults that had been exposed to high levels during lung development. A limitation of our study was that respiratory symptoms and diseases were assessed by questionnaire, which may be affected by recall bias, leading to either over- or underestimation of the association. However, when lung function was evaluated and analyzed, the results of FEV₁ and FVC showed that 58% of children had a restrictive spirometric pattern. Similarly to our results, von Ehrenstein et al. (2005), found equivalent spirometry patterns in exposed adults who had a restrictive pattern and De et al. (2004),

reported in their study that 53% of exposed subjects to arsenic had restrictive lung disease. The restrictive pattern in our study was more frequently observed in the two most exposed group of children (65.2% and 60.5%) and it was positively associated with As urinary levels; additionally, DMA^V, first methylation, second methylation and %DMA^V levels were significantly higher in these Groups (3 and 4). On the other hand and when the severity grade of lung restriction was analyzed, the total arsenic levels were 2.3 times higher ($p<0.05$) among subjects with a moderate restrictive pattern when compared with those found in children with the low restrictive pattern; however this interpretation must be tempered because of the reduced number of children ($n=4$) with moderate restrictive pattern. Finally, we found that the first and second methylation levels, act as risk factors (OR 1.44 and 1.26; respectively) for abnormal spirometry pattern. According with the data previously mentioned our results suggest that long-term exposure to arsenic through drinking water increases the risk for abnormal lung function and for restrictive spirometric patterns.

Several authors have suggested that the mechanisms of action of As in the lungs is that it can enhance tissue inflammation (De et al., 2004; Nemery, 1990), induce respiratory function impairment by oxidative stress (Lantz and Hays, 2006) and/or produce or increase pulmonary fibrosis (Nemery, 1990; von Ehrenstein et al., 2005). These side effects may be due to arsenic accumulation in the lung (Bertolero et al., 1981; Marafante et al., 1981) mainly as methylated arsenicals (MMA^V and DMA^V).

Inflammation biomarkers

It has been proposed that a persistent and chronic inflammation modifies the airway remodeling process propitiating the development of chronic lung disease. The airway remodeling process has been linked to the equilibrium between the proteases as MMP-9 and its antiproteases or inhibitors (TIMP-1, RAGE) (Vignola et al., 2004).

RAGE levels in the lung are quite high, and its expression has been demonstrated on the basolateral surfaces of alveolar epithelial type I cells (Neeper et al., 1992). Olsen et al. (2008) has recently shown that chronic exposure of mice or humans to arsenic in drinking water alters bronchiolar lavage or sputum proteins levels. In our study, we found a significant decrease of 1.3 and 1.9 times the sputum sRAGE levels in volunteers from groups 2 and 3, respectively, when compared with that found in children from group 1; in addition, and after the adjustment for several covariables, sRAGE levels were negative and significantly associated with DMA^V, second methylation, %MMA^V and with %DMA^V. In a previous study, Lantz et al. (2007), reported As-induced alterations in sRAGE in humans. They carried out a study in which they evaluated 57 adults exposed to arsenic through drinking water, and found that the sputum sRAGE levels were negatively correlated ($p=0.01$) with total urinary inorganic arsenic levels. These authors did not find correlations between sRAGE levels with arsenic species, which differs from our study; this difference could be due to the level of arsenic exposure (higher in our work) and to the age of the studied subjects (children in our study). On the other hand, when we analyzed the sRAGE levels according to the spirometric pattern, a significant decrease of 1.3 times was registered in those subjects with the restrictive pattern. Supporting these results, reduction of RAGE levels have been reported in persistent systemic inflammation and oxidative stress which are

common features of airflow disruption and emphysema in the lungs (Gopal et al., 2014; Kumano-Kuramochi et al., 2009). Additionally, in a recent study, a decrease level of esRAGE (endogenous secretory soluble receptor for advanced glycation endproducts) and total sRAGE in plasma was reported in COPD patients when compared with the levels registered in controls. Moreover, in this previous study, a positive association was found between total sRAGE and FEV₁, FEV₁/FVC (Li J, Schmidt AM. 1997). Some studies have been carried out with the aim to try to explain the mechanisms that can potentially explain the airflow remodeling and reductions in sRAGE levels in the lavage and sputum samples after exposure to different xenobiotics. It has been suggested that arsenic may change RAGE gene expression by altering promoter region methylation or by affecting transcriptional regulators of RAGE (Queisser et al., 2008; Tohgi et al., 1999), and that the blockade or down-regulation and/or loss expression of RAGE resulted in impaired cell adhesion. Small interfering RNA-induced knockdown of RAGE increased cell proliferation and migration of A549 cells and human primary fibroblast *in vitro*, as well as its involvement in cell adhesion, appear to be processes linked to the onset and/ or progression of fibrosis pathogenesis (Liang et al., 2012). These results indicate that sRAGE serves a protective role in the lung, and that loss of the soluble form of the receptor is related to functional changes of pulmonary cell types, with the consequences of fibrotic disease (Finlay et al., 1997). This soluble form (sRAGE) helps to inhibit inflammatory by buffering binding of ligands to the membrane form of the receptor. So, when arsenic decreases the levels of sRAGE it allows more activation of the cellular associated receptors and an increased inflammatory response. In agreement with the previously mentioned, our data suggest that sRAGE plays an important protective role (OR 0.69, 95% CI 0.52-0.99) in pulmonary function because sRAGE levels were significantly higher in children with normal spirometric pattern than in those with an abnormal pattern.

Matrix metalloproteases (MMPs) are a family of neutral proteases that play a vital role throughout the wound healing process; in the regulation of the inflammatory response (Finlay et al., 1997) increasing evidence suggests a key role for at least some of the MMPs in the development of chronic and acute lung disease (Ohnishi et al., 1998; Tanaka et al., 2000; Okada et al., 1992). As part of the lung inflammatory response, proteolytic enzymes including the MMPs are continually secreted in the airways. The activity of the MMPs is regulated by metalloproteinase inhibitors, with TIMP-1 considered to be the major inhibitor (Achanzar et al., 2002; Murphy and Willenbrock, 1995) and both have been considered as sensitive markers of lung inflammation in humans (Josyula et al., 2006). Arsenic exposure has been associated with changes in MMP-9 in prostate cells (Achanzar et al., 2002), keratinocytes (Cooper et al., 2004) and in humans (Josyula et al., 2006). In our work, in contrast with the relationship between sRAGE and arsenic exposure levels, we did not find a dose-response between MMP-9 or MMP-9/TIMP-1 with arsenic, but MMP-9 levels were significantly increased in Group 2, and MMP-9/TIMP-1 concentration was slightly higher in Group 3 ($p>0.05$); the last increase is not an effect on MMP-9 but is dependent on a decrease in TIMP-1 levels, therefore, it is expected that arsenic exposure leads to increased proteolytic activity with the subsequent side effects on the airway remodeling process in the lung. In the adjusted regression model, a positive and significant association was found between MMP-9 and MMP-9/TIMP-1 with the second methylation and with %DMA^v. At

present only one study evaluating the association between arsenic exposure and sputum inflammatory markers has been published. Indeed, Josyula et al. (2006), in a total of 73 subjects with a mean age over 60 years exposed to arsenic at relatively low concentrations (~ 20 mg/L) found a positive association between sputum MMP-9/TIMP-1 with urinary arsenic concentration. This appeared to be due primarily to a decrease in TIMP1 rather than an increase in MMP-9. The different results found between Josyula et al. (21), and our current study, could be due to the different number of subjects evaluated (bigger in our study), to the dissimilar mean age (children in our study) and/or to the level of exposure to arsenic (higher in our study). In another study assessing the relationship between arsenic exposure and serum MMP-9 levels, the authors found an association between increased environmental arsenic exposure and increased concentration of serum MMP-9. Both previous studies and ours, show a dysregulation in the synthesis/secretion of these lung inflammatory markers induced by arsenic exposure.

Conclusion

Our data indicates that chronic arsenic exposure modifies negatively sRAGE, MMP-9 and MMP-9/TIMP-1 levels, and increases significantly the frequency of an abnormal spirometric pattern in children. It is important to establish these modes of action so that preventive programs aimed at abolishing pulmonary diseases in children and later in adulthood can be developed.

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Table 1
Socio-demographic, anthropometric characteristics and life style of the participants

Results are shown as arithmetic mean, standard deviation and percent.

	Group 1 n=69	Group 2 n=69	Group 3 n=69	Group 4 n=69
Age (years)	9.1±1.81	9.23±1.87 *	9.04±1.75	8.88±1.62 *
Gender (%)				
Female	56.99	59.78	50.54	52.17
Male	43.01	40.22	49.46	47.83
BMI **	18.3±3.84	18.2±4.56	17.5±3.75	17.3±3.44 *
Second hand smoking (%)				
No	73.86	80.49	78.57	63.53
Yes	26.14	19.51	21.43	36.47
Type of water used at home (%)				
Purified	59.34	51.11	35.56	28.89 *
Tap water	38.46	46.67	63.33	68.89
Both	2.20	2.22	1.11	2.22
Type of water used at school (%)				
Purified	21.35	20.22	20.00	23.08
Tap water	76.40	77.53	77.78	75.82
Both	2.25	2.25	2.22	1.10
Type of water for cooking (%)				
Purified	18.8	16.4	14.5	8.8 *
Tap water	76.8	82.0	82.6	91.1
Both	4.3	1.4	2.9	0

T test, Mann Whitney test or Chi²

* p<0.05 (Groups 2-4 vs Group 1)

** BMI: calculated as: weight/height²

Table 2
Urinary and drinking water arsenic levels by study group

Results are shown as arithmetic mean and standard deviation.

	Group 1 n=68	Group 2 n=69	Group 3 n=69	Group 4 n=69
Total arsenic (µg/g)	43.12±14.8	89.62±16.3 *	151.03±21.6 *	311.40±129.4 *
As ^{III}	4.62±3.35	9.55±4.67 *	18.44±9.43 *	42.46±21.6 *
As ^V	12.46±15.45	9.97±19.79	7.56±14.63	9.12±5.87 **
MMA ^v	5.37±2.47	11.38±4.53 *	20.88±7.15 *	43.52±22.64 *
DMA ^v	21.59±16.58	55.70±23.37 *	98.39±24.97 *	204.52±87.66 *
First methylation	0.85±0.89	0.85±0.42	0.93±0.31 *	0.94±0.33 *
Second methylation	4.38±3.63	5.38±2.63	5.23±1.94	5.02±1.45
iAs	17.21±15.32	19.30±18.28	25.75±16.39 *	52.56±25.03 *
%iAs	39.77±33.20	22.03±21.30 **	17.32±12.12 **	16.71±5.33 **
%MMA ^v	12.42±3.51	12.64±4.25	13.81±4.11 *	13.94±3.14 *
%DMA ^v	48.90±33.1	61.75±22.44	65.35±15.14	66.25±9.68
As in drinking water (µg/L)	71.8±47.6	96.8±30.4 *	106.3±52.7 *	137.2±93.8 *

Ttest or Mann Whitney.

*
p=0.001

**
p<0.05 (Groups 2-4 vs Group 1)

Table 3
Spirometric pattern and severity of pulmonary restriction in the studied population

Results are shown as percentage.

	Group 1 (n=69)	Group 2 (n=69)	Group 3 (n=69)	Group 4 (n=68)
Spirometric pattern				
Normal	42.85	40.0	31.88	39.43
Restrictive	55.55	55.38	65.21	60.56
Obstructive	1.5	4.61	2.89	0
Severity of pulmonary restriction				
Low (< 70)	57.14	53.84	66.66	54.92
Moderate (< 60 <70)	0	0	0	5.63
Moderate to severe (< 50 <60)	0	1.53	0	0

T-test, Mann Whitney or Chi² * < 0.05 (Groups 2-4 vs Group 1)

Table 4
Lung inflammatory biomarkers by studied groups

Results are shown as arithmetic mean and standard error.

	Group 1 (n=69)	Group 2 (n=69)	Group 3 (n=69)	Group 4 (n=68)
sRAGE (pg/mL)	491.0±45.8	344.9.0±53.4 ^{**}	320.9±76.1 ^{**}	475.5±54.8
MMP-9 (ng/mL)	215.6.0±36.5	334.2±48.5 [*]	314.7±36.0	177.0±28.2
TIMP-1 (ng/mL)	92.2±8.8	105.8±12.9	95.8±7.5	94.3±8.5
MMP-9/TIMP-1	11.8±3.8	10.4±2.7	24.5±12.6	9.9±2.6

Ttest, Mann Whitney or Chi²

^{*}
p<0.05

^{**}
p< 0.01 (Groups 2-4 vs Group 1).

Table 5
Arsenic and inflammation biomarkers levels by spirometric patterns

Results are shown as arithmetic mean and standard deviation.

	Normal Pattern (n=115)	Restrictive Pattern (n=160)
Total As ($\mu\text{g/g}$)	142.5 $\pm$ 117.8	151.0 $\pm$ 123.5
As ^V	12.6 $\pm$ 17.2	8.0 $\pm$ 13.0 *
DMA ^V	91.4 $\pm$ 87.4	95.4 $\pm$ 80.8 **
First methylation	0.8 $\pm$ 0.4	0.9 $\pm$ 0.5 **
Second methylation	4.7 $\pm$ 2.6	5.1 $\pm$ 2.5 **
%DMA ^V	57.7 $\pm$ 26.6	61.9 $\pm$ 20.3 **
sRAGE (pg/mL)	499.3 $\pm$ 457.4	377.2 $\pm$ 407.8 **
MMP-9 (ng/mL)	251.8 $\pm$ 281.3	232.5 $\pm$ 265.5
TIMP-1 (ng/mL)	89.8 $\pm$ 63.9	98.4 $\pm$ 66.0
MMP-9/TIMP-1	18.7 $\pm$ 60.2	6.9 $\pm$ 15.51 *

Ttest, Mann Whitney or Chi²

*
p<0.05

**
p<0.01; (restrictive pattern vs normal pattern)

Table 6

Crude and adjusted Odds Ratios for selected variables and the risk for abnormal spirometric pattern.

	<u>Crude</u>			<u>Adjusted</u> [*]		
	OR	p	95%CI	OR	p	95%CI
Second methylation	1.170	0.017	1.04-1.31	1.160	0.015	1.02-1.30
%DMA	1.012	0.033	1.00-1.02	1.013	0.026	1.00-1.02
sRAGE	0.999	0.032	0.99-0.99	0.999	0.029	0.99-0.99
MMP-9/TIMP-1	0.985	0.026	0.97-0.99	0.986	0.049	0.97-0.99

* Adjusted by: frequent sibilance, type of fuel used for cooking, mothers' schooling and number of glasses of water drank per day.

Table 7

Multivariate analysis between the lung inflammatory biomarkers and arsenic species levels.

Ln sRAGE[*]	β	95% CI	P
ln DMA ^v	-0.101	-0.18 -0.017	0.018
First methylation	-0.349	-0.54 -0.15	0.0001
%MMA ^v	-0.501	-0.95 -0.046	0.001
%DMA ^v	-0.175	-0.29 -0.05	0.004
Ln MMP-9^{**}			
Second methylation	0.20	0.05 0.34	0.007
%DMA ^v	0.21	0.06 0.35	0.005
Ln MMP-9/TIMP-1^{&}			
Second methylation	0.23	0.09 0.37	0.001
%DMA ^v	0.24	0.09 0.38	0.001

* Adjusted by: type of fuel used for cooking, waste burning close to house, number of glasses of water drank per day.

** Adjusted by: place of pregnancy, number of pulmonary diseases per year, mothers' schooling.

& Adjusted by: place of pregnancy, frequent sibillance, mothers' schooling.