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Arsenic methylation capacity is associated with breast cancer in northern Mexico

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ABSTRACT

Exposure to environmental contaminants, dietary factors and lifestyles may explain worldwide different breast cancer (BC) incidence. Inorganic arsenic (iAs) in the drinking water is a concern in many regions, such as northern Mexico. Studies in several countries have associated the proportion of urinary monomethylarsenic (%MMA) with increased risks for many As-related diseases, including cancer. To investigate the potential relationships between the risk of BC and the capacity to methylate iAs, a hospital-based case–control study (1016 cases/1028 controls) was performed in northern Mexico. Women were directly interviewed about their reproductive histories. The profile of As metabolites in urine was determined by HPLC-ICP-MS and methylation capacity was assessed by metabolite percentages and indexes. Total urinary As, excluding arsenobetaine (TAs-AsB), ranged from 0.26 to 303.29 μg/L. Most women (86%) had TAs-AsB levels below As biological exposure index (35 μg/L). Women with higher %MMA and/or primary methylation index (PMI) had an increased BC risk (%MMA $OR_{Q5vs.Q1} = 2.63$; 95%CI 1.89,3.66; p for trend <0.001; PMI $OR_{Q5vs.Q1} = 1.90$; 95%CI 1.39,2.59, p for trend <0.001). In contrast, women with higher proportion of urinary dimethylarsenic (%DMA) and/or secondary methylation index (SMI) had a reduced BC risk (%DMA $OR_{Q5vs.Q1} = 0.63$; 95%CI 0.45,0.87, p for trend 0.006; SMI $OR_{Q5vsQ1} = 0.42$, 95%CI 0.31,0.59, p for trend <0.001). Neither %iAs nor total methylation index was associated to BC risk. Inter-individual variations in iAs metabolism may play a role in BC carcinogenesis. Women with higher capacity to methylate iAs to MMA and/or a lower capacity to further methylate MMA to DMA were at higher BC risk.

Introduction

Breast cancer (BC) incidence is increasing worldwide with an estimated 1.38 million new cancer cases diagnosed in 2008 (Ferlay et al., 2010). Differences in environmental conditions throughout the world may be related to the wide BC incidence range among countries; however, information about specific environmental exposures and BC risk is limited, contradictory or absent (IOM, 2012). In the particular case of Mexico, BC incidence and mortality are concentrated in the states located along the US–Mexico border, where incidence rate is about 60% higher than in rest of the country (Palacio-Mejia et al., 2009). Fifty eight percent of BC cases occur in Mexican women under 54 years of age (Knaul et al., 2009), but only about a third of them have the high risk reproductive profile for BC (i.e. early age of menarche, nulliparity, late age at first birth, etc.) (Lopez-Carrillo et al., 1997). It is possible that contrasting exposures to environmental contaminants, dietary factors and lifestyles may contribute to explain the differences in BC incidence and mortality between northern and central-southern Mexico.

Arsenic (As) is a natural element of the earth's crust that occurs in the groundwater and surface water of many parts of the world. Arsenic is a recognized human carcinogen since epidemiological studies have shown that inorganic As (iAs) exposure is associated with cancer of the lung, skin, and bladder, and possibly with kidney, and liver tumors (ATSDR, 2007; IARC, 2012). The International Agency for Research on Cancer (IARC) has classified arsenic as a group 1 carcinogen (IARC, 2012). For many years, the presence of As in the drinking water of some areas of northern Mexico has been a major concern (Cebrián et al., 1994). Arsenic levels have ranged in Sonora from 71 to 305 μg/L

Abbreviations: BC, breast cancer; iAs, inorganic arsenic; TAs, total As; AsB, arsenobetaine; MMA(III), monomethylarsonous acid; MMA(V), monomethylarsonic acid; DMA(III), dimethylarsinous acid; DMA(V), dimethylarsinic acid; PMI, primary methylation index; SMI, secondary methylation index; TMI, total methylation index; GM, geometric means; OR, odds ratio; 95% CI, 95%: confidence interval; ODD, oxidative DNA damage.

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(Wyatt et al., 1998), Chihuahua from 15 to 300 μg/L (Camacho et al., 2011) and Región Lagunera from 7 to 600 μg/L (Del Razo et al., 1990).

Arsenic is metabolized in many organisms, including humans, by a series of reduction and methylation reactions. Arsenate is reduced to trivalent arsenite, then oxidatively methylated to monomethylarsonic acid (MMAV), reduced to monomethylarsonous acid MMA(III), methylated to dimethylarsinic acid (DMAV) and then reduced to dimethylarsinous acid DMA(III) (Thomas et al., 2001). In humans, some As remains in its inorganic form, which is readily excreted in urine along with its metabolites. In general terms, the biomethylation has long been thought to be a major detoxification process, as the pentavalent methylated metabolites are less reactive towards cellular macromolecules and are eliminated more rapidly. However, the intermediates MMA(III) and DMA(III) are highly toxic and may be partially responsible for As toxicity. Methylation capacity shows large inter-individual variations; typically ingested iAs is excreted as 10–30% iAs, 10–20% MMA, and 60–70% DMA (Vahter, 2002).

Previous studies have suggested that altered profiles of As species in urine reflect inter-individual differences in the efficiency of iAs metabolism and may determine individual cancer susceptibility. As compared to controls, subjects showing higher iAs, MMAV and its percentage (%MMA), and/or primary methylation index (PMI = MMA/iAs), as well as lower (DMAV) and its percentage (%DMA), and/or secondary methylation indexes (SMI = DMA/MMA) have higher risks of skin (Hsueh et al., 1997; Yu et al., 2000; Chen et al., 2003a), bladder (Chen et al., 2003b; Steinmaus et al., 2006; Pu et al., 2007; Huang et al., 2008) and lung (Steinmaus et al., 2010) cancer. Most of these studies were performed in populations exposed to As levels in drinking water above 200 μg/L (Steinmaus et al., 2006); however, limited information is available at lower exposure levels (Pu et al., 2007).

The available information regarding potential associations between As and BC incidence is scarce. In a prospective Danish cohort, a small but significantly increased risk for BC (incidence rate ratio = 1.05; 95% CI, 1.01–1.10) was reported in association with As exposure via drinking water (time-weighted average); however, no significant associations were found with cancers of the lung, bladder, liver, kidney, prostate, colorectal, or skin melanoma. The average As exposure for the cohort ranged between 0.05 and 25.3 μg/L (mean = 1.2 μg/L) (Baastrup et al., 2008). An ecological study in Argentina found no association between BC cases reported to a local cancer registry and As levels in ground water (ND-330 μg/L) (Aballay et al., 2012). More recently, García-Esquinas et al. (2013) reported that low to moderate exposure to inorganic As was prospectively associated with increased cancer mortality from lung, prostate, and pancreas, but not from BC in American Indians.

Experimental evidence has shown that sodium arsenite induced ROS generation, DNA oxidative damage, heme oxygenase, metallothionein and c-Myc proteins, NF- B activation along with decreased methylenetetrahydrofolate reductase (MTHFR) levels and cell proliferation in human BC MCF-7 cells (Ruiz-Ramos et al., 2009a,b). More recently, chronic As exposure was shown to drive human breast epithelial cells (MCF-10A) into a cancer cell phenotype through overexpression of aromatase, thereby activating oncogenic processes independent of ER (Xu et al., 2014). These findings suggest that As has the ability of inducing proliferation in normal and transformed breast epithelial cells.

To date no study has reported on the potential association between iAs metabolism and BC risk. Therefore, a hospital-based case–control study was conducted to investigate the potential relationships between the risk of BC and the capacity to methylate iAs, as assessed by the profile of urinary As metabolites.

Material and methods

Study population. A hospital-based case–control study was performed from 2007 to 2011 in northern Mexico (Coahuila, Chihuahua, Durango, Nuevo León, and Sonora). Incident cases were identified from main public tertiary hospital units in the study area population ($n = 17$), including Health Department (Secretaría de Salud), Social Security (Instituto Mexicano del Seguro Social), and State Workers' Social Security (Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado) hospitals, as well as university health centers. A total of 1016 patients with histopathologically confirmed BC were identified, with the following inclusion criteria: minimal age of 18, without any other cancer history, and with a residency period of >1 year in the study area. Controls were 1028 healthy women, with no history of cancer, with a residency period >1 year in the same residence zone, matched by five years age with the index case. They were identified through the master sample framework used for the National Health Surveys from which a probabilistically selected list of housing addresses along with an access sketch to facilitate their location was obtained (Tapia-Conyer et al., 1992). In the houses where there was more than one eligible woman, only one participant was randomly chosen. Conversely, if no eligible woman was found in a household, or if she declined participation in the study, a new home was systematically located according to the standardized survey procedures. A grocery incentive was given to controls to increase the response rate. This study was approved by the Mexico National Institute of Public Health ethics committee.

Interviews. Information about sociodemographic characteristics; clinical, reproductive and family medical history, and dietary habits were obtained by face-to-face interviews to women who gave their informed consent. Their height and weight were also obtained. Patients were interviewed after their diagnosis, before any kind of treatment (average time from diagnosis to interview ~2 months). All participants were blinded to the study hypothesis. The response rates (participants/eligible) were 93.7% for cases and 99.7% for controls. The main reason given by the small proportion of women (71/2115; 3.36%) who refused participation in the study was lack of interest. There were no significant differences regarding age and years of education between participating and non participating women.

Urine samples. A first morning void urine sample was collected from each woman in a sterile disposable polypropylene urine collection cup. In all cases, urine samples were obtained before any cancer treatment was performed (including surgery and radiation therapy). An aliquot of 4 mL of urine was prepared in a Cryovial (Simport Scientific, Beloeil, QC, Canada) and stored frozen at or below −20 °C and then at −70 °C at CINVESTAV in Mexico City where creatinine was determined and samples were prepared for shipping to the University of Arizona to be analyzed for As.

Arsenic determination. Urinary concentrations (μg/L) of species As(III), As(V), MMA(V), DMA(V), and arsenobetaine (AsB) were determined by high-performance liquid chromatography ICP-MS system in The Analytical Section of the Hazard Identification Core at the University of Arizona according to Gilbert-Diamond et al. (2011). Measurements below the detection limit were imputed their corresponding value [As(III): 0.12; As(V): 0.20; MMA(V): 0.12; DMA(V): 0.08; AsB: 0.08] divided by two. Creatinine was determined by spectrophotometry using a commercial kit (Randox Creatinine Kit) with 1 mg/dL as DL, according to a previously described methodology (Blanco-Munoz et al., 2010).

Smoking and alcohol. Smoking was evaluated as the product of total years of smoking by the number of cigarette packs smoked per day (1 pack = 20 cigarettes). Duration was the number of years between the starting age and the age at which smoking stopped, or the age at interview for subjects still smoking.

Alcohol intake was estimated with a semi-quantitative food frequency questionnaire. Women were queried about the consumption

of beer, wine, brandy, rum, and tequila. The ethanol content in drinks was estimated in grams, using the Food Intake Analysis System (FIAS) Millennium Edition software (The University of Texas School of Public Health, TX, USA), with the following equivalencies: one drink (12 fl oz can/bottle) of beer = 12.96 g; one drink (3.5 fl oz wine glass) of white wine = 9.58 g; one drink (1 fl oz wine glass) of red wine = 2.74 g; and one drink of rum, brandy or tequila (1.5 fl oz 1 jigger) = 14.03 g of ethanol.

Statistical analysis. Arsenic exposure was characterized in the study population through geometric means (GM) and standard deviations and 10, 50 and 90 percentile urinary concentrations of total As (TAs), As species, and iAs methylation capacity indexes. Total As was calculated by using both the sum of iAs, MMA, DMA plus or minus AsB concentration (TAs or TAs-AsB, respectively). iAs was calculated as the sum of trivalent (AsIII) and pentavalent (AsV). %iAs, %MMA and %DMA species were estimated using in the denominator TAs-AsB or TAs. Inorganic As methylation capacity was estimated by the indexes corresponding to the primary (PMI = MMA/iAs), secondary (SMI = DMA/MMA) and total (TMI = DMA/iAs) methylation indexes. Creatinine concentration was added as a separate independent variable in the multivariate models to adjust for urine dilution, as suggested by Barr et al. (2005). The distributions of TAs and its metabolites in the groups under study were improved by means of a log transformation, and their GM were compared with *t*-tests.

To identify potential confounding variables, correlation coefficients between log transformed percentages of As metabolites and methylation capacity ratios with: TAs-AsB (μg/L), AsB (μg/L), creatinine (mg/dL), age (years), age at menarche (years), parity (number), age at first pregnancy (years), total breastfeeding (months), body mass index (kg/m^2), smoking (pack-years) and alcohol (g/week) were estimated among controls. Those variables that resulted significantly correlated with at least one of the six As markers (%iAs, %MMA, %DMA, PMI, SMI, and TMI) were considered as covariables in further multivariate models.

Using logistic regression models, the associations between BC and the percentage of each As species and methylation capacity ratios were estimated according to the observed quintile distribution among controls. Based on the median value of each quintile a lineal trend was assessed accordingly. All analyses were performed with the software Stata 12 (StataCorp, CollegeStation, TX, USA).

Results

By design the mean age of cases and controls (~54 years) was not significantly different. The average residence period in the study zone was also similar, for cases and controls (~51 years). About one third of women were premenopausal. As expected, cases were significantly more likely to experience menarche at a younger age than controls (12.91 vs. 13.02 years), have fewer or no children (3.98 vs 5.31 children), have an older age at first birth (22.48 vs. 20.16 years), and have a shorter period of breastfeeding (30.75 vs. 59.94 months). A significantly higher proportion of cases (35.69%) were overweight (BMI 25.00–29.99), as compared to controls (32.91%). In addition, 28.09% of all women were ever smokers (~3 pack-years on average) and 17.29% reported alcohol consumption (cases vs. controls = 2.63 vs. 0.46 g per week) (data not included in tables).

In the entire population, TAs ranged from 0.37 to 1677.19 μg/L (0.45 to 7047.25 μg/g creat), while TAs-AsB values ranged from 0.26 to 303.29 μg/L (0.32 to 1683.83 μg/g creat). Most cases (90.06%) and controls (82.68%) had TAs-AsB levels under 35 μg/L, the biological exposure index (BEI) for iAs plus methylated metabolites in urine (ACGIH, 2004). The contribution of AsB to TAs was significantly higher in the cases (6.57%) than in the control group (4.23%) (data not included in tables). As expected, the GM of %MMA were significantly higher in the cases than in the controls, whereas those of %DMA were significantly lower, and there were no significant differences in %iAs. Moreover, PMI was significantly higher among cases, whereas SMI and TMI were lower. Controls had significantly higher TAs, TAs-AsB, iAs, MMA and DMA GM creatinine corrected (μg/g creat) levels than cases. No significant differences were found in creatinine or AsB levels (Table 1).

Among controls, age at menarche and parity did not correlate significantly with the percentages of As species nor with methylation capacity ratios. In contrast, TAs-AsB, AsB, creatinine, age, age at first pregnancy, lifetime breastfeeding, BMI, smoking and alcohol showed different correlation patterns (Table 2).

Women with higher %MMA showed an increased BC risk ($OR_{Q5vs.Q1}$ = 2.63; 95% CI 1.89–3.66; *p* for trend <0.001). In contrast, women with higher urinary %DMA ($OR_{Q5vs.Q1}$ = 0.63; 95% CI 0.45–0.87; *p* for trend 0.006) had a reduced BC risk; BC risk was not associated with %iAs. Regarding methylation capacity indexes, women with higher PMI values had an increased BC risk ($OR_{Q5vs.Q1}$ = 1.90, 95% CI 1.39–2.59, *p* for trend <0.001). In contrast, women with higher SMI values (OR_{Q5vsQ1} = 0.42, 95% CI 0.31–0.59, *p* for trend <0.001) had a reduced BC risk; BC risk was not associated with TMI (Table 3). The ORs for BC and TAs or TAs-AsB were 0.77 (CI 0.71–0.84) and 0.69 (CI 0.63–0.77), respectively (data not included in tables).

Discussion

The main finding of this study was that BC risk was associated with alterations in the efficiency of iAs metabolism. There was an increased %MMA accompanied by a decreased proportion of DMA in BC cases. Women with higher capacity to methylate iAs to MMA (PMI) and/or with lower capacity to further methylate MMA to DMA (SMI) showed a higher BC risk.

Our findings are in agreement with epidemiologic studies performed in several countries, which reported increased cancer risks (skin, bladder and lung) in populations exposed to high As concentrations in drinking water and presenting changes in the profile of iAs metabolites in urine (IARC, 2012). For skin cancer, our findings agreed with the positive association with PMI and negative with SMI, at As exposures leading to average urinary TAs levels of 43 μg/L (Chen et al., 2003a). For urothelial cancer, with the findings of a Taiwanese hospital-based case–control study performed in populations without a history of high As exposure and exposed to low levels in drinking water (ND-4.0 μg/L) leading to urinary TAs levels of ~30 μg/g creat; urothelial cancer was positively associated with %MMA and PMI, but negatively with %DMA and SMI (Pu et al., 2007). Our findings were also in agreement with a recent study performed in Argentina whose results showed an association between %MMA and lung cancer at TAs-AsB concentrations ranging from 4.8 to 112.3 μg/L and a history of high As exposure; however, no data on the concentrations of urinary As metabolites in cases and control individuals were presented (Steinmaus et al., 2010). A more recent case–control study performed in northern Chile confirmed the elevated lung and bladder cancer risks in subjects with higher %MMA values (Melak et al., 2014). In these studies performed on populations exposed to either low or high concentrations of As in drinking water, the increased %MMA in urine accompanied by reduced %DMA was consistently associated to an increased cancer risk.

In the present study, urinary TAs concentration was higher in controls than in BC cases, yielding a negative association between TAs or TAs-AsB and BC. This was in agreement with the negative association observed with %DMA, since DMA is the major component of TAs in urine. A possible explanation is that cancer patients metabolized As less efficiently than their controls, as previously observed in epidemiological studies where %MMA(V) was significantly associated with skin and bladder cancer, without significant differences in urinary TAs levels between cases and controls (Yu et al., 2000; Chen et al., 2003a,b). Cancer patients may have higher demands on methylation pathways and cofactors which may account for a diminished TAs excretion, probably due to

Table 1
Urinary arsenic characterization in the study population.

Arsenic variables[a]	Cases ($n = 1016$)		Controls ($n = 1028$)		*P* value*
	GM ± GSD	Median (P10–P90)	GM ± GSD	Median (P10–P90)	
Creatinine (mg/dL)	59.79 ± 2.19	65.71 (20.98–153.99)	57.00 ± 2.40	63.99 (18.49–161.5)	0.194
Corrected urinary arsenic (μg/g creat)					
iAs	1.57 ± 2.66	1.44 (0.50–5.75)	2.23 ± 3.08	1.95 (0.63–10.01)	<0.001
MMA	1.61 ± 2.95	1.57 (0.43–6.35)	2.12 ± 3.10	1.82 (0.58–9.39)	<0.001
DMA	10.97 ± 3.07	10.98 (3.30–40.48)	17.16 ± 3.03	15.62 (4.87–75.73)	<0.001
AsB	1.36 ± 9.12	1.15 (0.09–28.65)	1.26 ± 10.69	0.85 (0.08–38.29)	0.483
TAs	20.63 ± 3.07	19.03 (5.48–87.53)	29.82 ± 3.36	25.90 (7.17–153.40)	<0.001
TAs-AsB	15.04 ± 2.68	14.33 (4.58–52.24)	22.35 ± 2.89	19.96 (6.40–100.04)	<0.001
Arsenic species percentages					
iAs	10.42 ± 1.90	10.18 (5.05–22.89)	9.98 ± 1.76	10.15 (5.39–18.87)	0.107
MMA	10.68 ± 1.51	10.80 (6.60–17.4)	9.49 ± 1.51	9.83 (5.64–15.28)	<0.001
DMA	72.94 ± 1.39	78.11 (64.34–86.18)	76.79 ± 1.24	79.70 (68.01–87.40)	<0.001
Arsenic methylation capacity indexes					
PMI (MMA/iAs)	1.03 ± 1.95	1.11 (0.41–2.22)	0.95 ± 1.77	1.00 (0.5–1.79)	0.006
SMI (DMA/MMA)	6.83 ± 1.86	7.09 (3.79–12.68)	8.09 ± 1.7	8.00 (4.52–14.88)	<0.001
TMI (DMA/iAs)	7.00 ± 2.45	7.62 (2.78–16.95)	7.69 ± 2.07	7.84 (3.64–16.12)	0.009

GM, geometric mean; GSD, geometric standard deviation; P10, 10th percentile, P90, 90th percentile; TAs, total arsenic; TAs-AsB, total arsenic minus arsenobetain; PMI, primary methylation index; SMI, secondary methylation index; TMI, total methylation index.
[a] The percentage of samples with detectable levels of As(III), As(V), MMA(V), DMA(V) and AsB was 80.74%, 43.68%, 98.05%, 99.51%, and 75.68% among the controls, and 71.15%, 43.80%, 96.06%, 99.51 and 79.72% among the cases, respectively.
* *p* value for *t*-test.

genetic and nutrition differences not considered in this study, such as intake of methyl donors, (i.e, folate, methionine, betaine, etc.) and/or genotype differences in the genes involved in As metabolism. Therefore, an altered metabolism in BC patients might result in the formation of As species with a strong tissue binding ability, thus reducing the amount of As excreted in urine, as described in laboratory animals (Vahter, 2002), and suggesting that %MMA is a better biomarker for As toxicity than TAs.

The molecular mechanisms of As carcinogenicity are still poorly understood. The generally accepted mechanisms are the induction of structural and numerical chromosomal abnormalities and the potential involvement of altered epigenetic regulation in gene expression. Aberrant DNA methylation is an early event in carcinogenesis that activates oncogenes and silences tumor suppressors promoting proliferation of abnormal cells. However, it is likely that interrelated genetic and epigenetic mechanisms together contribute to the toxicity and carcinogenicity of As (Ren et al., 2011). Recent studies performed in As methylation-proficient and -deficient cell lines have provided evidence supporting the relationship between oxidative DNA damage (ODD) and iAs metabolism, in particular that methylation to MMA causes ODD and that MMA(III) causes ODD without further methylation (Kojima et al., 2009; Tokar et al., 2014). These findings support the idea that the intermediate trivalent and highly toxic MMA in the tissues may be the primary toxic species of ingested iAs (Bredfeldt et al., 2006).

It is widely accepted that As methylation capacity varies according to reproductive, environmental and genetic differences among individuals. iAs methylation capacity has been shown to increase during breastfeeding and pregnancy, possibly due to the effects of estrogen on the synthesis of choline and methionine required for iAs methylation and/or the intake of folic acid and other B vitamins (Tseng, 2009). In the present study, cumulative breastfeeding was negatively associated with %MMA but not with PMI, and positively with SMI, but not with %DMA in control women. Given that breastfeeding is a well-known protective factor for BC (WCRF/AICR, 2007), it was considered a covariable, but no significant differences with non-adjusted models were observed.

A significant negative correlation between methylation capacity (increased %iAs and reduced %DMA, SMI and TMI) as alcohol consumption increased was also found, but a significant increase in PMI with smoking. In view that inconsistent results have been reported regarding the effects of smoking and alcohol on iAs methylation (Tseng, 2009) and that these factors have been related to BC risk (WCRF/AICR, 2007; Collishaw et al., 2009), they were considered as covariables in the adjusted models, but there were no significant differences with non-adjusted models.

Our results also showed significant negative associations between BMI and %MMA and PMI, but positively with SMI values. The direction of the association between BMI and iAs methylation efficiency reported here is in agreement with previous studies (Tseng, 2009; Gomez-Rubio et al., 2011). Given that BMI is considered a BC risk factor in postmenopausal women, with opposite effects in premenopausal women (WCRF/AICR, 2007), our multivariate models were adjusted by this variable and further stratified by menopausal status, and the main results remained significant, except for %DMA among premenopausal women (data not shown).

Table 2
Correlation coefficients between percentages of urinary arsenic species and methylation capacity indexes and selected potential confounders in the control group.

Selected variables	Correlation coefficients					
	Arsenic species percentages[a]			Methylation capacity indexes		
	iAs	MMA	DMA	PMI	SMI	TMI
TAs-AsB (μg/L)	−0.17*	0.10*	0.14*	0.24*	−0.02	0.17*
AsB (μg/L)	−0.30*	−0.19*	0.13*	0.16*	0.20*	0.27*
Creatinine (mg/dL)	−0.02	0.15*	0.03	0.13*	−0.10*	0.03
Age (years)	−0.20*	−0.10*	0.13*	0.12*	0.13*	0.19*
Age of menarche (years)	−0.01	0.05	−0.01	0.05	−0.04	0.01
Parity (number)	−0.04	−0.05	0.02	0.01	0.05	0.04
Age at 1st pregnancy (years)[b]	0.04	0.08*	−0.04	0.02	−0.08*	−0.04
Lifetime breastfeeding (months)	−0.04	−0.08*	0.02	−0.01	0.07*	0.04
BMI (kg/m^2)	−0.03	−0.24*	0.03	−0.15*	0.20*	0.03
Smoking (pack-years)	−0.04	0.03	−0.03	0.06*	−0.03	0.03
Alcohol (g per week)	0.08*	0.05	−0.07*	−0.05	−0.07*	−0.08*

SD, standard deviation; TAs, total arsenic; TAs-AsB, total arsenic minus arsenobetain.
[a] Using TAs-AsB in the denominator.
[b] Among parous women.
* $p < 0.05$.

Table 3
Breast cancer odds ratios in relation to percentages of urinary arsenic species and methylation capacity indexes.

Arsenic variables	Odds ratios (95%CI)[a]						
	Continuous[b]	Quintiles[c]					
		1st	2nd	3rd	4th	5th	*P* for trend[d]
Arsenic species percentages[e]							
iAs							
N cases/controls	1016/1028	212/206	166/206	220/205	189/206	229/205	
Model A	1.13 (0.97,1.30)	1.00	0.78 (0.59,1.04)	1.04 (0.80,1.37)	0.89 (0.68,1.17)	1.09 (0.83,1.42)	0.255
Model B	1.02 (0.86,1.22)	1.00	0.78 (0.57,1.06)	0.99 (0.73,1.33)	0.84 (0.61,1.15)	0.93 (0.68,1.27)	0.944
Model C	1.00 (0.84,1.19)	1.00	0.77 (0.56,1.05)	0.97 (0.71,1.31)	0.83 (0.60,1.13)	0.90 (0.65,1.24)	0.815
MMA							
N cases/controls	1016/1028	126/206	162/206	218/205	221/206	289/205	
Model A	2.03 (1.63,2.53)	1.00	1.29 (0.95,1.74)	1.74 (1.30,2.33)	1.75 (1.31,2.35)	2.30 (1.73,3.06)	<0.001
Model B	2.26 (1.75,2.90)	1.00	1.42 (1.02,1.98)	1.95 (1.41,2.70)	2.01 (1.45,2.79)	2.76 (1.99,3.83)	<0.001
Model C	2.15 (1.67,2.77)	1.00	1.35 (0.96,1.88)	1.88 (1.36,2.61)	1.89 (1.36,2.62)	2.63 (1.89,3.66)	<0.001
DMA							
N cases/controls	1016/1028	251/206	239/206	192/205	183/206	151/205	
Model A	0.44 (0.29,0.67)	1.00	0.95 (0.73,1.24)	0.77 (0.59,1.01)	0.73 (0.56,0.96)	0.60 (0.46,0.80)	<0.001
Model B	0.53 (0.34,0.81)	1.00	0.96 (0.72,1.28)	0.84 (0.62,1.13)	0.78 (0.58,1.06)	0.61 (0.44,0.85)	0.003
Model C	0.56 (0.37,0.86)	1.00	0.97 (0.73,1.30)	0.86 (0.64,1.16)	0.80 (0.59,1.08)	0.63 (0.45,0.87)	0.006
Methylation capacity indexes							
PMI							
N cases/controls	1016/1028	201/206	151/207	165/204	204/206	295/205	
Model A	1.22 (1.06,1.40)	1.00	0.75 (0.56,1.00)	0.83 (0.62,1.10)	1.01 (0.77,1.34)	1.47 (1.13,1.92)	<0.001
Model B	1.44 (1.21,1.70)	1.00	0.85 (0.62,1.16)	1.03 (0.75,1.42)	1.17 (0.86,1.60)	1.88 (1.38,2.56)	<0.001
Model C	1.43 (1.21,1.69)	1.00	0.83 (0.60,1.14)	1.03 (0.75,1.41)	1.15 (0.84,1.57)	1.90 (1.39,2.59)	<0.001
SMI							
N cases/controls	1016/1028	293/206	216/206	216/205	164/206	127/205	
Model A	0.58 (0.49,0.69)	1.00	0.74 (0.57,0.96)	0.74 (0.57,0.96)	0.56 (0.43,0.73)	0.44 (0.33,0.58)	<0.001
Model B	0.56 (0.47,0.68)	1.00	0.70 (0.53,0.93)	0.75 (0.56,1.00)	0.53 (0.39,0.72)	0.40 (0.29,0.56)	<0.001
Model C	0.58 (0.48,0.71)	1.00	0.70 (0.53,0.94)	0.77 (0.57,1.03)	0.54 (0.40,0.73)	0.42 (0.31,0.59)	<0.001
TMI							
N cases/controls	1016/1028	231/206	188/206	231/205	165/206	201/205	
Model A	0.87 (0.78,0.96)	1.00	0.81 (0.62,1.07)	1.00 (0.77,1.31)	0.71 (0.54,0.94)	0.87 (0.67,1.15)	0.293
Model B	0.92 (0.81,1.05)	1.00	0.92 (0.68,1.24)	1.10 (0.82,1.48)	0.82 (0.60,1.12)	1.03 (0.75,1.42)	0.970
Model C	0.94 (0.83,1.07)	1.00	0.93 (0.69,1.26)	1.12 (0.84,1.51)	0.83 (0.61,1.14)	1.06 (0.77,1.46)	0.842

[a] Model A: crude model; Model B: adjusted for log transformed TAs-AsB (μg/L), AsB (μg/L), age (years), BMI (kg/mt^2), total breastfeeding (months), and alcohol (g per week), and original raw values for smoking (pack-years) and age at first pregnancy (<19, 19–21, >21, nulliparous); Model C: further adjusted for log transformed creatinine (mg/dL).
[b] Log transformed variables.
[c] Based on the control group distribution, quintile cut-offs were as follows: iAs% (≤6.55, >6.55–8.75, >8.75–11.27, >11.27–14.90, and >14.90), MMA% (≤7.01, >7.01–8.92, >8.92–10.79, >10.79–13.30, and >13.30), DMA% (≤72.37, >72.37–77.81, >77.81–81.38, >81.38–84.96, and >84.96), PMI (≤0.65, >0.65–0.89, >0.89–1.12, >1.12–1.48, and >1.48), SMI (≤5.55, >5.55–7.10, >7.10–9.01, >9.01–11.73, and >11.73), and TMI (≤4.88, >4.88–6.82, >6.82–9.29, >9.29–13.01, and >13.01, respectively).
[d] *p* for trend across quintile medians.
[e] Using TAs-AsB in the denominator.

It has also been proposed that methylation of iAs becomes limited at certain levels of iAs exposure, possibly due to the inhibition of the second methylation step by the excess iAs (Styblo et al., 2000; De Kimpe et al., 1999). However, several epidemiological studies have investigated the impact of increasing As exposure on iAs methylation capacity with inconsistent results; most of them have reported increased %MMA, but some reported increased %DMA whereas others have shown negative associations (Tseng, 2009), suggesting that the relationships between TAs and DMA have not been as consistent among studies as those with MMA. In the present study, TAs-AsB levels were negatively correlated with %Asi and positively with %MMA and %DMA. Therefore, to reduce the confounding effect of TAs-AsB on the relationship between iAs methylation efficiency and BC risk, we added TAs-AsB as a covariable in the multivariate models. In addition, we also measured urinary AsB levels to be included as a covariable in the models, reducing the possibility of confounding by the DMA potentially originating from AsB and seafood (Lai et al., 2004; Longnecker, 2009).

Some methodological features of this study should be considered to interpret our results. Urine samples were obtained before cancer treatment to exclude the possibility that treatment could influence iAs methylation. However, we measured urinary methylation products after disease status was ascertained; therefore, we could not exclude its influence on the urinary iAs methylation pattern, as discussed for similar studies (Steinmaus et al., 2006). Measurement of As and its metabolites in urine is the most common strategy for assessing human exposure and is an integral measurement of exposure; however, a single measurement does not allow evaluation of cumulative exposure or exposure windows, unless a fairly constant iAs exposure via drinking water is assumed. We assessed total MMA(III + V) as a surrogate for MMA(III), since the highly toxic trivalent forms are very unstable in urine; therefore, the association of MMA and BC here reported is potentially conservative due to a bias towards the null value.

Based on the regression model proposed by Calderon et al. (1999), we estimated that 60% of the women under study were exposed to As levels in drinking water above the current WHO maximum contaminant level of 10 μgAs/L (WHO, 2011), and 34% above the current limit (25 μgAs/L) in Mexico (Secretaría de Salud, 2000). Action oriented measures are required to reduce As exposure in Mexico, and should consider the harmonization of the Mexican current maximum contaminant level of As with that recommended by WHO, in conjunction with the development of alternative strategies, such as dietary interventions, aimed to prevent and/or counteract As exposure health effects (Gamble et al., 2006; Hall and Gamble, 2012).

In conclusion, to our knowledge, this is the first study that reports an increased BC risk associated to As methylation capacity. The significant associations here reported were observed at As levels near the current BEI (35 μg/L) for iAs plus methylated metabolites in urine. Further studies are required to confirm if As exposure at levels leading to TAs-AsB

concentrations under the current BEI for As in urine and alterations in iAs metabolism are risk factors for BC.

Conflict of interest

The authors declare that there are no conflicts of interest

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