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Exposure to metals and congenital anomalies: A biomonitoring study of pregnant Bedouin-Arab women

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HIGHLIGHTS

- Almost a third of the pregnant women had a detectable metal in their urine.
- Aluminum and Arsenic were the most prevalent metals in urine.
- The study investigates pregnant women exposed to a hazardous environment.
- Household exposure was most highly associated with the detection of metals in urine.
- Impact is specific to a metal: Al–anomalies, As - preterm birth and lower weight.

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ABSTRACT

Background: The Bedouin-Arab population in Israel comprises a low socio-economic society in transition. Smoking among males and consanguineous marriages are frequent. A previous study showed elevated rates of major malformations within groups from this population residing near an industrial park, where high ambient values of arsenic (As) and nickel (Ni) were detected, compared to groups living in remote localities.
Objectives: We estimated the extent of exposure to metals in pregnant Bedouin-Arab women in relation to congenital malformations.
Methods: We collected maternal urine samples from 140 Bedouin women who gave birth in a local hospital. Patient medical history, type of marriage (consanguineous or non-consanguineous), and parental exposure history were collected by interview and medical records.
Results: Aluminum (Al) was detected in 37 women (26.4%), cadmium (Cd) in 2 (1.4%), As in 10 (7.1%), and Ni in 1 woman (0.7%). The detected rate of Cd exposure was low, though more than 92% of the fathers reported smoking. Concentrations of Al were higher for women residing within 10 km of the local industrial park (Prevalence Ratio (PR) = 1.12, p-value = 0.012) or who reported using a wood burning stove (PR = 1.37, p-value = 0.011) and cooking over an open fire (PR = 1.16, p-value = 0.076).
Exposure to Al was adversely associated with minor anomalies (OR = 3.8, p-value = 0.046) after adjusting for history of abortions (OR = 6.1, p-value = 0.007). Fetuses prenatally exposed to As were born prematurely (p-value = 0.001) and at lower weights (pv = 0.023).
Conclusions: The study population of pregnant women is exposed to high levels of metals mainly of household origin. Our findings may be generalized to similar populations in developing countries.

1. Introduction

The Bedouin-Arab population in Israel is characterized by a high rate of congenital malformations in comparison to the country's Jewish and

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other Arab populations (Bentov et al., 2006). In southern Israel, the Bedouin-Arab population numbers around 200,000 inhabitants, most of who are of low socio-economic level owing to their high rate of unemployment and generally low education level (The Central Bureau of Statistics in Israel). Consanguineous marriages, i.e., marriages within a family between first, second or third cousins, are estimated at 45% (Jaber et al., 1994; Jaber and Halpern, 2006). Half of the Bedouin population resides in traditional tribal settlements, where they live in temporary shacks or tents that do not afford sufficient protection from ambient air pollution. In addition, cooking and heating is often by open fire. While about 90% of Bedouin men smoke, the habit is much less common (10%) among Bedouin women (Jaber et al., 1994).

Being essentially unprotected from outdoor pollution, the Bedouin-Arab population is potentially exposed to the emissions of the local industrial park (IP), which comprises 24 chemical and pharmaceutical facilities and an incinerator, and which serves as the principal industrial waste disposal site for the entire country. The list of emissions from the chemical plants and evaporation pools includes a variety of aliphatic, aromatic and polycyclic hydrocarbons and a few dozen nonorganic agents, including heavy metals. The 2010 periodic report based on monitor readings of IP emissions showed high values for arsenic (As) and nickel (Ni) (Ecological Laboratories, 2012). The report also showed the presence of cadmium (Cd) and aluminum (Al), which in combination with other elements have known toxicological effects and are detrimental to human development. The presence of other measured chemical elements, e.g., lead, was minimal.

A study in southern Israel in 2006 indicated increased rates of major congenital malformations (MCMs) among Bedouin-Arab newborns whose mothers lived within 20 km of an IP when compared to Bedouin-Arab newborns residing in remote localities (5.6% vs. 4.8%, respectively, $p < 0.01$), suggesting an adverse impact of IP proximity on the levels of MCMs observed in the Bedouin population (Sarov et al., 2003). In this study, differences in the rates of MCMs as a function of distance were unlikely to be explained by the utilization of different health care services or by variations in the rates of consanguineous marriages.

The etiology of malformations is poorly understood and is believed to be a product of independent effects or a combination of genetic factors, medications, behavioral, occupational and/or environmental exposures (Wigle et al., 2008). The pregnancy period deemed the most sensitive and with the most potential for the induction of birth defects has been identified as 3–8 weeks of gestation (Mattison, 2010), but during the second trimester, the fetus is also considered highly sensitive to environmental exposure (Lacasana et al., 2005; Kim et al., 2007).

Developmental diseases of the embryo, among which are neural tube defects (Brender et al., 2006), have been related to the exposure to heavy and semi-heavy metals, as shown mostly in animal models (Lopez et al., 2008; Martinez et al., 2004; Robinson et al., 2009, 2010). Sources of exposure to metals include industry, the home environment, health behavior and nutritional and dietary habits. Exposure to heavy metals can be assessed using epigenetic channels (Ho et al., 2012). Specifically, heavy metal exposure is believed to cause oxidative stress that, in turn, may explain the future development of chronic and acute disorders (Cortessis et al., 2012). Some examples of birth defects in humans related to exposure to metals were found in studies reporting the adverse effects of tobacco on fetal development, partially due to the exposure to the toxic heavy metals contained in cigarette smoke (Rogers, 2009). An ecological study in China, where the distribution of geophysical elements in soil, water and food was compared between a province with an unusually high rate of birth defects and a control area, indicated that metals, specifically higher sulfur and lower strontium and aluminum levels, were associated with congenital malformations (Yu and Zhang, 2011).

The association of congenital malformations with the exposure to metals in pregnancy has been based mostly on ambient assessments of contaminated air distribution, but not on individual, biological measurements. The current study is the first attempt to assess the contribution of prenatal exposure to heavy and semi-heavy metals in pregnancy (further referred as "metals") to a clinical expression of malformations diagnosed at birth.

2. Objectives

We aimed to 1) investigate individual concentrations of metals in a population of pregnant women of Bedouin-Arab origin, and 2) analyze the association of metal levels in maternal urine at delivery with environmental exposures during pregnancy and congenital malformations and other birth outcomes in their fetuses and newborn infants.

We hypothesized that adverse environmental exposures will increase levels of metal concentrations in urine and that the increased exposure to metals at the time of the study will be associated with higher neonatal morbidity.

3. Methods

We focused our investigation on Cd, Ni, Al and As. Ni and Al were chosen for investigation based on the above-mentioned periodic report (Ecological Laboratories, 2012), whereas Cd was chosen due to the high prevalence of smoking among Bedouin-Arab males. We included As because it may be released from the open fires used for cooking and heating and due to the widespread use of pesticides among the Bedouin. Although lead was previously linked as a risk factor for congenital anomalies (Brender et al., 2006), it was not tested in the current study in view of its relatively low levels according to an official government report that reviewed the extent of lead pollution in Israel in the 1990s (Foner, 1999) and to a study of lead levels in teeth conducted around the same time (Bercovitz et al., 1993). The overall low level of lead in the country corresponds with the severe restrictions put in place, in the form of local regulations, for the use of this chemical in the color and toy industries.

3.1. Study population

During June–December 2013, we enrolled women upon their arrival to the Obstetric Emergency Department (OED) of the Soroka University Medical Center in Beer-Sheva, a tertiary medical center in southern Israel where all the births in the region take place. We included mothers of Bedouin-Arab origin who resided in Bedouin-Arab localities in the Negev. Only singleton deliveries were included. Newborns/fetuses were excluded if: (a) their gestational age was under 22 weeks; (b) birth weight was under 500 g; (c) delivery took place on the way to the hospital; (d) conditions precluded collection of a sterile urine sample, e.g., bleeding or rupture of membranes prior to arrival in OED; or (e) maternal age < 18 years.

Eligible women were approached by a trained, Arabic-speaking interviewer in the OED during the day shift on working days. Women were provided with explanations about the study and were invited to consent to participate in the study.

3.2. Sample collection and testing

Spot urine samples were collected in sterile 120-mL specimen containers. All urine samples were maintained at 4 °C for a maximum of 48 h and later frozen at −20 °C until shipment to the toxicology laboratory at the Sheba Medical Center in Tel-Hashomer, Israel, for analysis. To increase testing efficiency, we sampled mothers of fetuses with malformations, which artificially increased the proportion of anomalies analyzed in the study. Moreover, if the study hypothesis proves to be true, i.e., malformations are associated with exposure to metals, the values of metals in urine will be overestimated to a certain extent in our study.

3.3. Sample analysis

The samples were quantitatively evaluated in the toxicological laboratory at the Sheba Medical Center using graphite furnace atomic absorption spectroscopy (Perkin Elmer AA800).

3.4. Data collection

An interview performed after delivery included questions on exact address during pregnancy, socio-economic status, family history of malformations, consanguineous marriages, parental exposure to environmental or occupational hazards, health behavior during pregnancy and recent medical problems. We collected information on maternal co-morbidities and diagnoses during pregnancy and delivery. Assessment of neonatal clinical characteristics was available for analysis based on the data collected in an Admission-Transfer-Discharge (ATD) database of the SUMC.

3.5. Diagnosis of congenital malformations

Diagnosis of malformations in a newborn or stillbirth fetus was made upon delivery or after birth according to ICD-9 and defined as major or minor depending on a clinical definition of a defect and its impact on the child's health and based on European Surveillance Congenital Anomalies (EUROCAT) (European Surveillance of Congenital Anomalies) as presented in the Appendix A (Table 1). Ante-partum death (APD) and peri-partum death (PPD) were classified as the highest level of outcome severity. The category of major malformations was assigned to fetuses with at least one major malformation. The mildest degree of severity was assigned to fetuses with only minor malformations.

3.6. Statistical analysis

Data were entered on EpiData software and analyzed using the SAS 9.2 package (SAS Institute). Continuous variables were presented as mean ± standard deviation (sd), median, and extreme values, and they were compared using *t*-test, analysis of variance or the Wilcoxon test, depending on the distribution type of the variable. Categorical variables were presented as proportions of non-missing values and compared by Chi-square or Fisher's exact test. The significance level used in the analysis was set at 5%.

The association of exposure to metals with a diagnosis of malformations was performed by logistic regression, which provided an adjusted odds ratio (OR) estimation. Due to the relatively small sample size, the cut-off for significance level in the multivariable analysis was set at 10%.

3.6.1. Analysis of analytes

All subjects with detected concentrations of metals were defined as subjects with metals above the limit of detection (LOD). We calculated geometric means, 95% confidence intervals and fiftieth percentile for urinary metal concentrations. To estimate a level of exposure to metals in the study population, concentrations below the LOD for an analyte were replaced by LOD/sqrt(2) (Baccarelli et al., 2005). In a univariate analysis, we compared geometric means of urinary metal concentrations between demographic and clinical subgroups using a ratio *t*-test on a lognormal distribution. Multivariate analysis of factors affecting exposure to metals was investigated by a linear regression with a log-transformed concentration of analyte as the dependent variable. The exponent of the regression coefficients represents the estimates of the prevalence ratio (PR).

4. Results

Our study population comprised 140 pregnant women from whom spot urine samples were collected and tested for Cd, Ni, Al and As at delivery. Al tested above LOD in the urine of 37 women (26.4%), As in 10 women (7.1%), Cd in 2 women (1.4%) and Ni in 1 (0.7%). Overall, metals were detected in 45 women (32.1%), five of who (3.6%) were exposed to two metals concomitantly (Table 1). The following analysis focused on Al and As because they were the most frequently detected metals. In women with metals above LOD, the geometric means of concentrations of Al and As were 12.2 μg/L (95%CI: 10.9; 12.6) and 37.6 μg/L (95%CI: 21.0; 67.3), respectively.

Interviews were performed on 81.4% of the women enrolled in the study (114/140). The women who were not interviewed did not differ by demographic characteristics, distance from the IP, or detection of metals, but their offspring weighed on average 300 g less than the offspring in the group on whom the questionnaire data were collected.

Between the exposure subgroups, there were no differences between study participants in terms of age, parity or rate of consanguineous marriages (Table 2). Women were on average 28.0 ± 6.2 years old. About 30% of the participants were prima para, and 33% of them have experienced at least five deliveries in the past (data not shown). Consanguineous marriages were frequent (45%), and 81% of these were between first-degree relatives. Around 14% of women (19/140) were found to be “lacking perinatal care” (LOPC). None of the women reported smoking during pregnancy. The distribution of chronic comorbidities was similar between subgroups by exposure to metals. History of abortions was more frequent among women with concentrations of metals above LOD, but that finding was borderline significant for some: 17.6% in the group with any metal present (p-value = 0.056), 15.6% in the group with Al (p-value = 0.104), and 66.7% in the group with As (p-value = 0.011), compared to only 4.3% in women not exposed to metals. Infertility treatment and preeclampsia were found to be more frequent in groups exposed to metals, but the latter

Table 1
Urinary concentrations (ug/L) of metals in 140 study participants.

Metal	LOD, μg/L	% > LOD (n/140)	Within the population above LOD			Within the study population[a]	Normal range, μg/L	% > maximal normal value (n/140)
			Minimal and maximal values, μg/L	50th percentile, μg/L	Geometric Mean (95%CI), μg/L	Geometric Mean (95%CI), μg/L		
Aluminum	9.0	26.7% (37)	7.2[b];28.3	11.0	12.2 (10.9;12.6)	7.6 (7.1;8.0)	0–15[c]	6.4% (9)
Arsenic	20.0	7.1% (10)	11.6;131.5	44.2	37.6 (21.0;67.3)	15.2 (14.4;16.0)	0–35[d]	4.3% (6)
Cadmium	1.0	1.4% (2)	1.9;8.7	5.3	4.1 (–)	–	0–5[d]	0.7% (1)
Nickel	5.0	0.7% (1)	3.0[b];3.0	3.0	3.0 (–)	–	0–15[d]	0
Any of the 4 metals	–	32.1% (45)		–	–	–		

[a] Values of Al and As below LOD were imputed by LOD/sqrt(2).
[b] This range is also referred to as the Biological Exposure Index (BEI) by the American Conference of Governmental Industrial Hygienists.
[c] Wang ST, Pizzolato S, Demshar HP. Aluminum levels in normal human serum and urine as determined by Zeeman atomic absorption spectrometry. Journal of Analytical Toxicology Vol. 15, 1991 pp. 66–70.
[d] We refer to all values reported by the lab as “above LOD level”, including 2 concentrations (Al = 7.2 μg/L and Ni = 3.0 μg/L) that appear to be lower than laboratory defined LOD.

Table 2
Demographical and clinical characteristics, by exposure to aluminum and/or arsenic.

Patients characteristics	Patients without metals[a] N = 95 (N = 70)[b]	Patients with aluminum N = 37 (N = 32)[b]	p-Value[c]	Patients with arsenic N = 10 (N = 3)[b]	p-Value[c]	Patients with 2 metals[d] N = 5 (N = 3)[b]	p-Value[c]
Maternal age, years							
Mean ± SD (n)	27.6 ± 6.1 (95)	28.8 ± 6.0 (37)	0.311	29.8 ± 7.4 (10)	0.287	30.0 ± 6.7 (5)	0.383
Lack of perinatal care, % (n/N)	11.6% (11/95)	18.9% (7/37)	0.272	10.0% (1/10)	1.000	0.0% (0/5)	1.000
Consanguineous marriage, % (n/N)	46.4% (32/69)	37.5% (12/32)	0.518	33.3% (1/3)	1.000	33.3% (1/3)	1.000
Resides in direction of prevailing wind from IP, % (n/N)	22.2% (20/90)	37.1% (13/35)	0.114	10.0% (1/10)	0.684	20.0% (1/5)	1.000
Distance from IP, km							
Mean ± SD	26.1 ± 20.4	23.9 ± 9.9	0.684	19.4 ± 10.9	0.316	22.1 ± 9.5	0.834
Median (n)	23.8 (90)	26.4 (35)		22.5 (10)		24.3 (5)	
Distance from IP < 10 km, % (n/N)	5.6% (5/90)	11.4% (4/35)	0.265	30.0% (3/10)	0.031	20.0% (1/5)	0.284
Chronic disease, % (n/N)	3.2% (3/95)	0.0% (0/37)	0.559	20.0% (2/10)	–	20.0% (1/5)	1.000
Diabetes mellitus	0.0% (0/95)	0.0% (0/37)	–	10.0% (1/10)	0.521	0.0% (0/5)	–
Thyroid disorders	2.1% (2/95)	0.0% (0/37)	1.000	0.0% (0/10)	0.521	0.0% (0/5)	1.000
Chronic hypertension	1.1% (1/95)	0.0% (0/37)	1.000	10.0% (1/10)	0.182	20.0% (1/5)	0.098
Infertility treatment, % (n/N)	0.0% (0/95)	0.0% (0/37)	–	10.0% (1/10)	0.095	20.0% (1/5)	0.050
Preeclampsia, % (n/N)	1.1% (1/95)	5.4% (2/37)	0.190	10.0% (1/10)	0.182	20.0% (1/5)	0.098
Self-reported history of abortions, % (n/N)	15.7% (11/70)	21.9% (7/32)	0.576	66.7% (2/3)	0.080	33.3% (1/3)	0.421
Spontaneous	4.3% (3/70)	15.6% (5/32)	0.104	66.7% (2/3)	0.011	33.3% (1/3)	0.158
Planned	0.0% (0/70)	6.3% (2/32)	0.096	0.0% (0/3)	–	0.0% (0/3)	–
Paternal smoking, % (n/N)	92.4% (61/66)	93.8% (30/32)	1.000	66.7% (2/3)	0.242	100.0% (3/3)	1.000
Husband employed, % (n/N)	85.7% (60/70)	87.5% (28/32)	1.000	100.0% (3/3)	1.000	100.0% (3/3)	1.000
Factors at husbands' work, % (n/N)							
Noise	80.0% (56/70)	90.6% (29/32)	0.255	100.0% (3/3)	1.000	100.0% (3/3)	1.000
Vibration	54.3% (38/70)	68.8% (22/32)	0.198	100.0% (3/3)	0.251	100.0% (3/3)	0.251
Excessive heat	7.1% (5/70)	12.5% (4/32)	0.457	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Neonatal outcomes							
Male gender, % (n/N)	56.8% (54/95)	51.4% (19/37)	0.697	30.0% (3/10)	0.180	0.0% (0/5)	0.018
Preterm delivery, % (n/N)	3.2% (3/95)	2.7% (1/37)	1.000	40.0% (4/10)	0.001	40.0% (2/5)	0.019
Birth weight, gm							
Mean ± SD	3292.3 ± 557.1	3181.2 ± 477.6	0.290	2550.0 ± 812.3	0.002	2508.0 ± 560.6	0.007
Median (n)	3350.0 (95)	3245.0 (37)		2677.5 (10)		2645.0 (5)	
Large-to-gestational age, % (n/N)	6.5% (6/93)	8.3% (3/36)	0.709	0.0% (0/10)	1.000	0.0% (0/5)	1.000
Small-to-gestational age, % (n/N)	5.4% (5/93)	11.1% (4/36)	0.264	0.0% (0/10)	1.000	0.0% (0/5)	1.000
Any anomaly or APD, % (n/N)	15.8% (15/95)	29.7% (11/37)	0.071	6.25% (0/10)	1.000	20.0% (1/5)	1. 000
Minor anomalies only[e]	10.5% (10/95)	24.3% (9/37)	0.043	0.0% (0/10)	0.593	0.0% (0/5)	1.000
Major anomalies[e]	8.4% (8/95)	10.8% (4/37)	0.738	0.0% (0/10)	1.000	0.0% (0/5)	1.000
APD	1.1% (1/95)	2.7% (1/37)	0.484	10.0% (1/10)	0.182	20.0% (1/5)	0.098

[a] This group includes subjects without Al, As, Ni and Cd detected in urine.
[b] Number of questionnaires collected.
[c] Comparison with patients under LOD for all metals.
[d] The combination of 2 metals was as follows: Aluminum and Arsenic detected in three patients, Arsenic and Cadmium detected in one patient and Aluminum and Nickel detected in one patient.
[e] Division into major and minor anomalies depended on a clinical definition of a defect and its impact on the child's health and based on European Surveillance Congenital Anomalies (EUROCAT). Minor anomalies could occur in patients with major anomalies.

association showed only a trend (p-value = 0.098). Prevalence of paternal smoking was 92%. Mothers with high concentrations of Al tended to live closer to, and more frequently downwind of, the IP, but this finding was not statistically confirmed (Table 2).

Preterm delivery was more frequent in patients with As in their urine compared to patients without detected metals (40.0% vs. 3.2%, p-value = 0.001) (Table 2). In the group exposed to As, this difference was reflected by a shorter gestational age (p-value = 0.005) and lower birth weight (2550 ± 812) compared to unexposed participants (3292 ± 557, p-value = 0.002). Similar differences were shown for the group exposed to two metals. There were no male fetuses in the group with two metals in the urine compared to 56.8% in the unexposed group (p-value = 0.018).

Patients with Al above LOD had significantly more minor anomalies (24.3% vs. 10.5% in the non-exposed group, p-value = 0.043), and the overall count of any anomalies or APD tended to be higher in this group (29.7% vs. 15.8% in the unexposed group, p-value = 0.071). The group with two metals had a high rate of APD (20%, calculated as 1/5 subjects compared to 1.1% in the non-exposed group, p-value = 0.098).

All women participating in the study reported living at the same addresses at least throughout their pregnancies, and over 94% were not working outside the home (Table 3). About 30% of the study population was living in tents or shacks with no difference as to their exposure to metals. Compared with participants in the non-exposed group, women with Al concentrations above LOD complained more frequently about untreated sewage spills (56.3% vs. 34.3%, p-value = 0.051) and the operation of transport vehicles in close proximity to their houses (78.1% vs. 45.7%, p-value = 0.003). All women with Al reported dust as a prominent and disturbing feature of their places of residence compared to 90.0% of the non-exposed women (p-value = 0.089). Women not exposed to metals reported more frequent use of air-conditioning for heating (p-value = 0.027), whereas participants in the group exposed to metals were more likely to use wood stoves for heating (17.6% vs. 2.9% in the non-exposed group, p-value = 0.014) and to cook on open fires (91.2% vs. 72.9%, p-value = 0.032). The group with Al above LOD reported using small water containers more frequently (71.9% vs. 50.0%, p-value = 0.052).

Living within 10 km of the local IP (PR = 1.39, p-value 0.012), use of wood stoves (PR = 1.37, p-value = 0.011) and cooking on open fires (PR = 1.16, p-value = 0.076) were independently associated with elevated concentrations of Al (Table 4).

Congenital malformations were found in 26 cases, of which 13.6% and 8.6% were diagnosed with minor and with major malformations, respectively. In addition, there were two cases of ante-partum death.

Table 3
Household sources of exposure of women by exposure to aluminum and/or arsenic.

Patients characteristics	Patients without metals[a] N = 95 (N = 70)[b]	Patients with aluminum N = 37 (N = 32)[b]	p-value[d]	Patients with arsenic N = 10 (N = 3)[b]	p-value[d]	Patients with 2 metals[c] N = 5 (N = 3)[b]	p-value[d]
Living in a shack/tent, % (n/N)	31.4% (22/70)	25.0% (8/32)	0.641	0.0% (0/3)	0.549	33.3% (1/3)	1.000
Works outside, % (n/N)	5.7% (4/70)	3.1% (1/32)	1.000	33.3% (1/3)	0.194	0.0% (0/3)	1.000
Disturbing environmental factors, % (n/N)							
Dust	90.0% (63/70)	100.0%(32/32)	0.095	66.7% (2/3)	0.298	100.0% (3/3)	1.000
Noise	5.7% (4/70)	9.4% (3/32)	0.675	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Waste	2.9% (2/70)	3.1% (1/32)	1.000	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Vegetation	1.4% (1/70)	6.3% (2/32)	0.231	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Sewage	34.3% (24/70)	56.3% (18/32)	0.051	66.7% (2/3)	0.287	66.7% (2/3)	0.287
Transport	45.7% (32/70)	78.1% (25/32)	0.003	100.0% (3/3)	0.105	66.7% (2/3)	0.595
Smell (frequently)	28.6% (20/70)	56.3% (18/32)	0.009	33.3% (1/3)	1.000	33.3% (1/3)	1.000
Type of Heating, % (n/N)							
Electric/Air-Conditioner	35.7% (25/70)	9.4% (3/32)	0.008	33.3% (1/3)	1.000	0.0% (0/3)	0.546
Oil stove with chimney	1.4% (1/70)	3.1% (1/32)	0.531	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Wood stove with chimney	2.9% (2/70)	18.8% (6/32)	0.011	0.0% (0/3)	1.000	0.0% (0/3)	1.000
Oil/gas stove, no chimney	41.4% (29/70)	56.3% (18/32)	0.201	66.7% (2/3)	0.571	66.7% (2/3)	0.570
Open Fire	17.1% (12/70)	12.5% (4/32)	0.770	0.0% (0/3)	1.000	33.3% (1/3)	0.450
Cooking on open fire, % (n/N)	72.9% (51/70)	93.8% (30/32)	0.016	100.0% (3/3)	0.563	100.0% (3/3)	0.563
Usage of Pesticides, % (n/N)	88.6% (62/70)	96.9% (31/32)	0.165	100.0% (3/3)	1.000	100.0% (3/3)	1.000
Usage of chemical fertilizer, % (n/N)	7.1% (5/70)	3.1% (1/32)	0.662	0.0% (0/3)	1.000	33.3% (1/3)	0.230
Using water containers, % (n/N)	91.4% (64/70)	96.9% (31/32)	0.429	100.0% (3/3)	1.000	100.0% (3/3)	1.000
Type of water containers used, % (n/N)							
Water tank	25.7% (18/70)	6.3% (2/32)	0.030	33.3% (1/3)	1.000	0.0% (0/3)	0.570
Small Containers	50.0% (35/70)	71.9% (23/32)	0.052	66.7% (2/3)	1.000	66.7% (2/3)	1.000
Barrel	15.7% (11/70)	18.8% (6/32)	0.777	0.0% (0/3)	1.000	33.3% (1/3)	0.421

[a] This group includes subjects without Al, As, Ni and Cd detected in urine.
[b] Number of questionnaires collected.
[c] The combination of two metals was as follows: Aluminum and Arsenic detected in three patients, Arsenic and Cadmium detected in one patient and Aluminum and Nickel detected in one patient.
[d] Comparison with patients under LOD for all metals.

Cardiovascular system anomalies and other systemic anomalies were the most common aberrations in the study (Appendix A, Table 1).

Above 90% of patients with minor malformations were males compared to 52.6% in the healthy sub-group (p-value = 0.006).

In the two mothers whose fetuses died in-utero, As was detected in one (50%) compared to in 7.9% of healthy patients (p-value = 0.058). Al was more common in mothers of newborns with malformations or APD cases compared to mothers of healthy newborns (42.3% vs. 22.8%, p-value = 0.029). Al concentration levels (>LOD) increased significantly with anomaly severity when comparing healthy births to minor anomalies and major anomalies or APD (PR = 1.10, p-value = 0.036).

The delivery of fetuses with minor anomalies was significantly associated with the detection of Al in maternal urine samples (OR = 3.8, p-value = 0.046) after adjustment for history of abortions, a factor strongly associated with anomalies (OR = 6.1, p-value = 0.007).

Table 4
Clinical characteristics and maternal exposure to metals by anomalies in fetuses.

Patients characteristics	Healthy patients N = 114	Patients with minor anomalies N = 12	Patients with major anomalies or APD cases N = 14	p-Value
Newborn characteristics				
Male gender, % (n/N)	52.6% (60/114)	91.7% (11/12)	42.9% (6/14)	0.021
Preterm delivery, % (n/N)	3.5% (4/114)	0 (0/12)	21.4 (3/14)	0.011
Gestational age, days				
Mean ± SD (n)	275.8 ± 14.6 (114)	276.6 ± 13.7 (12)	268.8 ± 27.7 (14)	0.990
Median	280.0	276.5	280.0	
Birth weight, g				
Mean ± SD (n)	3260.4 ± 506.0 (114)	3623.3 ± 387.4 (12)	2621.1 ± 841.7 (14)	<0.001
Median	3320.0	3722.5	2807.5	
LGA, % (n/N)	6.3% (7/111)	16.7% (2/12)	0.0% (0/14)	0.224
SGA, % (n/N)	2.7% (3/111)	0.0% (0/12)	42.9% (6/14)	<0.001
Any metal detected, % (n/N)	29.8% (34/114)	50.0% (6/12)	35.7% (5/14)	0.347
Aluminum detected, % (n/N)	22.8% (26/114)	50.0% (6/12)	35.7% (5/14)	0.090
Geometric Mean (95%CI)				
Within > LOD	11.7 (10.4; 13.2)	12.0 (8.4; 17.1)	15.4 (8.2; 28.9)	0.129[a]
Within population	7.3 (6.9; 7.7)	8.7 (6.8; 11.3)	8.7 (6.5; 11.8)	0.021[a]
Arsenic detected, % (n/N)	7.9% (9/114)	0.0% (0/12)	7.1% (1/14)	0.600
Geometric Mean within > LOD (95%CI)	42.9 (24.2; 75.9)	–	11.60	–
Cadmium detected, % (n/N)	1.8% (2/114)	0.0% (0/12)	0.0% (0/14)	0.793
Geometric Mean within > LOD (95%CI)	4.1 (–)	–	–	–
Nickel detected, % (n/N)	0.9% (1/114)	0.0% (0/12)	0.0% (0/14)	0.892
Geometric Mean within > LOD (95%CI)	3.00±. (1)	–	–	–

[a] The test compares geometric means between the 3 groups.

5. Discussion

The study showed wide exposure to semi-metals among the pregnant Bedouin-Arab women. Specifically, in 32.1% (24.4%–39.9%) of the women, at least one type of metal was detected, with 26.4% testing positive for Al followed by 7.1% for As. This study is the first to report the above findings in pregnant women and to do so using an individual biological test.

5.1. Household and industrial exposures and detection of Al and As

In line with our expectations, the detection of semi-metals in the women's urine was associated with adverse environmental exposures. Both industrial and household factors, such as residing within 10 km of the IP, the use of wood stoves for heating, and cooking over open fires, were related to the detection of aluminum in maternal urine. Arsenic was also more prevalent in women who resided close to the IP. However, to truly ascertain whether there is a connection between congenital anomalies and proximity to an industrial source will require a more rigorous assessment of ambient exposure, which is beyond the scope of this study, and therefore, it should be incorporated in future research.

5.2. Exposure to metals in relation to neonatal and maternal morbidity

Normal ranges of Al, As, Ni and Cd in urine for the general population have not been established, and therefore, standards applied in occupational medicine were used in the analysis, invariably causing a certain underestimation of exposure. Inspection of the actual values of metals in urine reveals that only 6.4% and 4.3% are above the maximal standard values of Al and As, respectively. However, we cannot diminish the significance of the potential adverse effect of this exposure when found in the sensitive population of pregnant women. Long-term exposure, reported as lifetime for most of the study population, may explain the observed effects on maternal and neonatal outcomes in the study. Thus, mothers exposed to metals were more likely to experience abortions, infertility treatments or preeclampsia. Also affected were their fetuses, in whom type of damage was generally specific to a certain metal. For instance, Al was associated with minor malformations, specifically, systemic aberrations such as cardiac and circulatory system anomalies. Even though the associations between exposure to Al and neonatal morbidity were expected, the effects of Al or its sources of exposure have not been sufficiently investigated. Toxicological research showed an adverse effect of Al on humans if consumed in a quantity of 1 μg per 1 kg of body weight (Yokel, 2012). Another study reported an excess of congenital anomalies among female workers at an aluminum smelter (Sakr et al., 2010).

The presence of As was related to preterm deliveries, lower weight and APD cases. In addition, exposure to As was associated with a lower prevalence of male fetuses, which in itself is an intriguing finding, but the relatively small numbers preclude drawing any substantive conclusions. Moreover, As is a known carcinogen linked to a variety of other morbidities, e.g., cardiovascular morbidity, that are outside the scope of the current study.

The possible mechanism of metals-induced morbidity has been explained as the result of oxidative stress. The extent of oxidative stress-induced damage may be controlled by gene expression regulation, shown to be an important component of cellular and/or tissue homeostasis (Chevrona and Costa, 2012), potentially explaining the variation in outcome severity.

Exposure to metals is frequently confounded by socio-economic level and medical care availability, but this may not be the case in our study. First, as stipulated by national law, maternal prenatal care is provided by accessible community clinics at no cost, and therefore, a variation in malformations is unlikely to be explained by a difference in the utilization of health care services, since they are accessible by everyone. Second, the specificity of an effect, such as Al being associated with malformations and As with other conditions, leads us to believe that these associations are not the result of residual confounding.

The highly toxic and carcinogenic Ni was detected in only one woman and Cd was detected in only two. The latter finding is surprising in view of the potentially high prevalence of exposure to secondhand smoke among Bedouin-Arab women considering that 92% of their husbands smoke. This finding is supported by a similar result by another study (Levine et al., 2013), which reported that urine samples from Arab-Israeli women had unexpectedly low levels of cotinine. Maziak et al. (2006) reported the same finding for Syrian women and explained it by the social customs of a traditional society, where females cannot freely mix with the men, and as such, their exposure to secondhand smoke was lower despite the high rates of smoking among their husbands.

Due to a certain bias in our sample in which we selectively included cases with malformations, we did not attempt a direct extrapolation of metal concentrations onto the population, even though the study participants were representative of the general population of Bedouin women in terms of age, parity and other demographic parameters.

5.3. Limitations

We were unable to obtain data on household exposure for 18.6% of the sample. Aside from the problem of small sample sizes for some of the subgroups, women who were not interviewed were usually discharged earlier than 48 h after birth or on a weekend. This may potentially cause a selection bias, as newborns of the women not interviewed (18.6%) weighed on average 300 g less than the newborns of mothers for whom the questionnaire was obtained (81.4%). However, this may not dramatically affect the results, as both groups had the same distribution of metals.

Concentrations of metals were estimated on the day of delivery, and therefore, they may not reflect the levels of exposure throughout the entire period of pregnancy. However, the half-lives of some of the elements are rather long, e.g., half-life estimates for Al is 7.7 years (Priest, 2004) and for Cd it is 10–35 years, (WHO) but they are relatively short for Ni, 17–48 h (Health Protection Agency), and As, 1–4 days (Department of Justice and Attorney-General, 2012). Assuming that no changes occurred in the women's household or environment (Ecological Laboratories, 2012), the levels measured at delivery may safely represent the exposures throughout the gestation.

To conclude, we showed wide exposure to metals in a susceptible population of Bedouin-Arab pregnant women, and these metals are likely to have adverse impacts in terms of maternal morbidity and neonatal outcomes. To the best of our knowledge, this is the first study to investigate the exposure to a metal biomarker in this vulnerable population, the members of which are also potentially exposed to very hazardous household environments. The low-socio economic level of the subjects in our study is likely to characterize the susceptible populations of many developing countries with similar household environments. Therefore, our findings may be generalized to other, similar populations, but the source of pollution is expected to vary. Furthermore, we showed that the possible impacts on health may be specific to the type of metal, i.e., Al was associated with malformations and As with chronic maternal health and preterm deliveries, findings that have been supported by animal studies and, usually indirectly, studies in humans. Our study also raised many questions and highlighted issues that require further, more in-depth investigation, which should be pursued in future research.

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