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ASSOCIATION BETWEEN PRENATAL EXPOSURE TO METALS AND NEONATAL MORBIDITY

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An association between prenatal exposure to (semi-)metals and of neonatal morbidity was assessed by introducing an oxidative stress as a possible intermediate step. An oxidative stress was measured by cell proliferation (CP) ratio in umbilical cord blood cells. Urine samples of 18 out of 58 enrolled women (31%) were positive for (semi-)metals; 25.9% of women were positive for aluminum (Al). The CP ratio was higher (1) in subjects with Al, (2) in mothers to newborns diagnosed as small-for-gestational age (p value = .052), (3) neonates that weighed less (p value = .079), and (4) in women who experienced repeated abortions (p value = .049). Our findings suggest the possibility of metal-induced oxidative stress.

Fetal development is the product of a long process of gestation during which time the organism is at risk of environmental disruptions, subsequently reflected later in a range of morbidity outcomes. Embryo-developmental diseases and other adverse birth outcomes have been related to exposure to heavy and semiheavy metals (Ferguson et al., 2013). Specifically, exposure to metals is believed to produce inflammation and oxidative stress, which may induce a future predisposition to chronic and acute disorders (Cortessis et al., 2012; Villamena, 2013). The aim of the current investigation was to explore the possible association between exposure to metals and an increased risk of morbidity by introducing an intermediate step of an oxidative stress (Figure 1). The cell proliferation (CP) ratio was measured in umbilical cord blood cells as an indicator of fetal oxidative stress (Lehmann et al., 2008).

Environmental exposure was shown to exert a modulating effect on fetal cell functioning in animals. Prenatal exposure to particulate matter (PM) modulated the fetal immune system and resulted in a postnatal immune dysfunction (Hong et al., 2013). Schaub et al. (2009) showed decreased lymphocytes proliferation in cord blood of mothers exposed to farming during pregnancy. However, the matrix of maternal exposure to chemicals and changes in cell has not been investigated extensively (Cooke 2014). Thus, the impact of prenatal exposure to metals on the level of CP was

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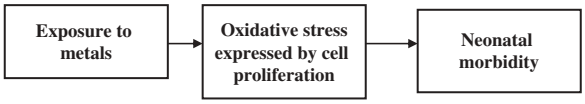

FIGURE 1. Suggested mechanism of the effect of metals on neonatal morbidity.

examined in umbilical cord blood, as well as the influence of CP on neonatal health.

METHODS

Women of Bedouin-Arab origin arriving for delivery at Soroka University Medical Center with a singleton fetus at gestational age >22 weeks and birth weight >500 g were enrolled. Spot urine specimens were collected in sterile 120-ml containers and maintained at –20°C until tested for aluminum (Al), cadmium (Cd), arsenic (As), and nickel (Ni) using graphite furnace atomic absorption spectroscopy (Perkin Elmer AA800 instrument). Samples of women experiencing rupture of membranes and/or bleeding were excluded to avoid contamination. Subjects with metals at concentrations above the limit of detection (LOD) were reported as positive for metals (i.e., Al $\geq$ 9 μgL; Cd $\geq$ 1 μgL; As $\geq$ 20 μgL; and Ni $\geq$ 5 μgL).

Assessment of oxidative stress was based on the CP test in red cells of umbilical cord blood (MTT cell proliferation assay [yellow MTT]). This test is widely used for assessing drug sensitivity, cytotoxicity, response to growth factors, and cell activation (Mosmann, 1983). A linear relationship between cell number and absorbance established for each cell enabled an accurate quantification of proliferation (Li, 2009).

Information on potential household exposures, demographic status, and other relevant factors was collected by a questionnaire processed during hospitalization after delivery. Comparisons between subgroups were conducted by Student's *t*- and Mann–Whitney tests for continuous variables and chi-squared or Fisher exact tests for categorical variables. Due to the small sample size, a significance level was set at $p < .1$ and no multivariable analysis was possible.

RESULTS

Urine samples were collected and CP tests performed on 58 women. Eighteen women (31%) were found positive for one of the 4 metals: 15 for Al (25.9%, geometric mean = 12 μg/L, range: 9–28.3 μg/L), 4 (6.9%) for As (range 14.1–75.5 μg/L), 1 for Ni (3 μg/L), and none for Cd. Two women were simultaneously exposed to two metals (Al and As, and Al and Ni). This study focused on the impacts of Al, as the most prevalent metal in the study population. The women were on average 28.4 ± 6.4 years old, while women with Al>LOD were older (30.8 ± 7.1) with more deliveries in the past (Table 1). There was no marked difference in the rate of consanguineous marriages or lack of prenatal care. Median CP ratio was 2.3, mean ± standard deviation 2.5 ± 1.2, range 0.9–6.4. The ratio was not associated with maternal age, but tended to be higher in subjects with Al detected, whereas 26.7% of them (4/15) had CP above the 90th percentile compared to 4.7% (2/43) in the group without Al (*p* value = .034). Newborns with CP above median were more likely to be diagnosed as small-for-gestational age (SGA) (*p* value = 0.052) and weighed less (p-value = 0.079). Of note, women with CP > 90th percentile experienced repeated abortions more frequently (33.3%) compared to others (3.9%, *p* value = .049). It is noteworthy that neonatal outcomes, including SGA, were similarly distributed between women positive and negative for Al. However, the presence of Al in urine seemed to enhance an association between elevated CP and SGA, as SGA diagnosis was present in 25% of newborns with CP > 90th percentile compared to none out of 11 newborns with lower CP.

DISCUSSION

Exposure to Al was statistically related to the 90th percentile of the CP ratio and with neonatal morbidity indices. Higher CP ratio was associated with a higher rate of SGA, as well as repeated abortions. Evidence indicates the

TABLE 1. Demographical Characteristics and Cell Proliferation Ratio by Aluminum Detection and Neonatal Morbidity

Demographical Characteristics and Cell Proliferation Ratio by Aluminum Detection, $n = 58$				
	Aluminum			
Women's characteristics	< LOD ($n = 43$)	> LOD ($n = 15$)	Total ($N = 58$)	p Value
Age, years				
Mean ± SD (n)	27.6 ± 6.1 (43)	30.8 ± 7.1 (15)	28.4 ± 6.4 (58)	.098
Min–max	20–42	20–41	20–42	
Parity				
Median (n)	2 (43)	5 (15)	4 (58)	.119
Min–max	1–11	1–12	1–12	
Lack of prenatal care, % (n/N)	11.6 (5/43)	20.0 (3/15)	13.8 (8/58)	.414
Cell proliferation ratio				
Mean ± SD (n)	2.4 ± 1.1 (43)	2.8 ± 1.4 (15)	2.5 ± 1.2 (58)	.311
Median	2.3	2.6	2.3	
Min–max	1.0; 6.4	0.9; 5.1	0.9; 6.4	
Above, % (n/N)				
50th percentile	48.8 (21/43)	53.3 (8/15)	50.0 (29/58)	.764
75h percentile	23.3 (10/43)	33.3 (5/15)	25.9 (15/58)	.502
90th percentile	4.7 (2/43)	26.7 (4/15)	10.3 (6/58)	.034
Neonatal Morbidity by 50th Percentile of Cell Proliferation Ratio, $N = 58$				
	Cell proliferation ratio			
Women's characteristics	<50th percentile ($n = 29$)	>50th percentile ($n = 29$)	Total ($N = 58$)	p Value
Preterm delivery, % (n/N)	3.5 (1/29)	0	1.7 (1/58)	1.000
Birth weight, g				
Mean ± SD (n)	3351 ± 463 (29)	3125 ± 427 (29)	3238 ± 456 (58)	.079
Min–max	2505–4195	2355–3870	2355–4195	
Small-for-gestational age, % (n/N)	0	17.2 (5/29)	8.6 (5/58)	.052
Large-for-gestational age, % (n/N)	10.3 (3/29)	3.5 (1/29)	6.9 (4/58)	.612

Note. LOD, limit of detection.

presence of altered cell functioning expressed as oxidative stress, which may partially explain the pathophysiological mechanism underlying exposure to metals. These findings are based upon objective, individually assessed mother–fetus biological measurements, thus increasing their validity. Our results support the recent review on the impact of environmental factors, which are frequently analyzed in a context of pathogenesis of current and future morbidity (Ho et al., 2012).

The CP assay used in the study is not the most comprehensive approach for establishing a metal-induced oxidative stress; however, it is related to a mitochondrial function that plays a key role in oxidative-stress generation in response to exogenous environments (Byun et al., 2013).

These conclusions need to be treated with caution due to some limitations:

- The small sample size precluded adjustments to possible confounding or utilization of path-analysis methodology.
- The study population features low socioeconomic level, reducing its generalizability.
- Exposure to metals was evaluated based on maternal urine. Testing for metals in umbilical cord would have resulted in more pronounced association estimates.
- The absence of association between metals and outcomes in this study weakens the grounds for our research. However, our results suggest that presence of Al may initiate pathophysiological processes in cells.

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