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Environmental uranium exposures and cytokine profiles among mother-newborn baby pairs from the Navajo Birth Cohort Study

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Abstract

The Navajo Nation was heavily mined for uranium (U) during the cold-war leading to a legacy of >1100 abandoned U mining, milling and associated waste sites. The Navajo Birth Cohort Study was initiated to assess the effect of non-occupational legacy exposure to U during pregnancy on birth outcomes and child development. We report that 92% of babies with detectable urine U at birth were born from mothers who had urine U concentrations greater than national norms during pregnancy, indicative of prenatal exposure to U. To assess immune alterations associated with U exposure on both mothers and babies, we investigated associations between cytokine profiles and maternal U and associations of these measures with cytokine profiles in babies. Effect sizes for the differences in cytokine profiles were more evident among babies than mothers. Overall, there were seven cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-10, GM-CSF, and TNF-α), for which the effect size for babies with higher than the national U concentrations was medium to large (ORs of 2.21 (1.08–4.52) through 1.71(0.76–3.83). In contrast, only three cytokines (IL-8, IL-12p70, and TNF-α) had effect sizes which almost reached medium strength (ORs of 1.64 (0.74–4.05) through 1.36 (0.65–2.87) in mothers with U above national norms. The effects of prenatal exposures to uranium and associated alterations in systemic immune responses resulting from U exposure could impact both maternal health as well as healthy child development through induction of inflammation, autoimmunity or other chronic diseases related to immune dysfunction that may affect long-term health.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2022.116292.

Keywords

Cytokines; Pregnancy; Newborn; Native Americans; Uranium Exposure

1. Introduction

For over 40 years, during the cold-war era, Tribal lands of the Navajo Nation were heavily mined for uranium (U) resulting in a legacy of >1100 abandoned U mining, milling operations, and associated waste sites (Brugge and Goble, 2002; Furlow, 2014; Navajo Nation AUM, 2007; Centers for Disease Control and Prevention Agency for Toxic Substances and Disease Registry, 2015). These abandoned and un-remediated mining and milling sites continue to pose an exposure risk to U and other co-occurring metals and metalloids such as arsenic to the communities living close to these sites (Hoover et al., 2017). Mixed metal contaminations were observed in drinking water, soils, homes, and structures that were built using remnant mining waste materials and structures left behind (Hoover et al., 2017). A geospatial analysis of the distribution of U and arsenic in unregulated water sources throughout Navajo demonstrated that both arsenic and U concentrations exceeded national drinking water standards in 15.1% (arsenic) and 12.8% (U) of tested water sources and that unregulated sources in close proximity (i.e., within 6 km) to abandoned U mines yielded significantly higher concentrations of arsenic or U than more distant sources (Hoover et al., 2017).

The Navajo Nation has long-standing concerns that exposures to legacy mining waste may be a contributing factor to poor health outcomes among tribal members, and effecting younger generations, especially infants and children (Hunter et al., 2015). The Navajo Birth Cohort Study, initiated in 2010 to address these concerns, has established that the median urine U concentrations were nearly three times higher than the NHANES median (Hoover et al., 2020; The Fourth National Report on Human Exposure to Environmental Chemicals Publication, 2015). Although U and arsenic exposures are linked to alterations in both cellular and humoral immune responses in animals (Dublineau et al., 2014; Burchiel et al., 2009; Xu et al., 2016), little is understood about the impact of these metals on immune system alterations that are associated with exposed populations to U mining-related waste materials (Gonzales et al., 2018; Hoover et al., 2019; Guéguen et al., 2017; Gazin et al., 2004; Li et al., 2014). Alterations in systemic immune responses resulting from mining waste exposure could lead to inflammation, autoimmunity or other chronic diseases related to immune dysfunction (Conrad and Mehlhorn, 2000; Lourenço et al., 2013). Population studies are key for understanding community-wide effects of metal exposures, the epidemiology of associated diseases, and ultimately steps for policy action. In addition, such studies in pregnant women lend important insight into the maternal-fetal immune environment, a key factor in the developmental origins of health and disease (Gluckman et al., 2010; Bauman and Van de Water, 2020). In this study, we determine associations between maternal exposure to U with maternal cytokine profiles and associations of both the maternal exposures and cytokine profiles on cytokine profiles in the babies.

2. Materials and methods

2.1. Study population and samples

The NBCS was initiated to assess non-occupational exposure to legacy U mining wastes among Navajo community members living on the Navajo Nation lands located in the southwestern United States (Hunter et al., 2015). Study participants were pregnant women between the ages of 15–45 years who had lived at least 5 years on the Navajo Nation. Recruitment and enrollment were conducted at participating Navajo Area Indian Health Service and PL-638 (Tribally-owned and managed) facilities and prenatal clinics after confirmation of pregnancy for all subjects. Between February 2013 and April 2017, 781 mothers and 764 babies were enrolled in the study with complete data available for 730 mothers. Blood and urine were collected from mothers at 36 weeks of the pregnancy or at delivery and from babies shortly after birth (within 24 to 36 h) in the nursery. Cord blood samples and matched maternal venous blood samples were collected at delivery from 52 mother-infant pairs for this analysis. All baby samples were using metal pre-screened baby urine bags provided by Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences of the National Center for Environmental Health (DLS CDC 2012–0036).

2.2. Serum cytokines measurements

Serum concentrations of 13 human cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, GM-CSF, IFN-γ and TNF-α) were quantified (pg/ml) in the serum of 52 matched cord blood and maternal venous blood samples using the EMD Millipore human 13-plex magnetic bead assay (HCYTOMAG-60 K, Sigma-EMD Millipore Inc., Burlington, MA). Manufacturer's guidelines were followed for assay protocol, standards and controls were included in each run. Detection levels and CV%s of each cytokine measured are provided in Supplemental Table 2. No differences in CV% were observed among samples from NBCS moms and babies. A MAGPIX multiplexing system with xPONENT software (Luminex, Austin, TX) was used for data acquisition. MILLIPLEX® Analyst 5.1 software was employed to determine concertation values for each cytokine in mother-baby pairs. LLOD concentrations (shown in Supplemental Table 2) were divided by square root of 2 in order to assign values due to left censoring (Croghan and Egeghy, 2003; Hornung and Reed, 1990).

2.3. Metal measurements

Urine U concentrations were determined by the urine multi-element method for Inductively Coupled Plasma-Mass Spectrometry (ICP-DRC-MS) at the Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences of the National Center for Environmental Health (DLS CDC 2012–0036). The detection limit (LOD) was reported as 0.002 μg/L (U.S. Department of Health and Human Services Centers for Disease Control and Prevention Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, 2019). Urinary U concentration values were acquired in microgram/l [μg/L] units but were converted to nanogram/l [ng/L] units to avoid rounding off errors during the analysis stage. Further, to maintain regression assumptions, the natural logarithm transformation was

applied on the U concentration values on their nanogram/l [ng/L] units plus one to offsets the shift at zero values.

Percentages of urine samples that were below the LOD of urinary U concentrations in NBCS moms were 9.6% and 48.1% in babies.

2.4. Statistical analysis

To describe categorical variables, frequency and relative frequencies were utilized. In addition, the McNemar's test was used to compare the proportion of exposure to U in matched pairs of mothers and babies. Medians, histograms, and scatterplots were used to describe numerical variables. Correlation analysis using Spearman correlation coefficients was also carried out. Spearman correlation was applied to log-transformed cytokine measurements from moms and babies due to some remaining skewed distributions of the concentrations.

To assess the linear association between U levels in moms and babies, a simple linear regression was employed, and to assess the median difference in cytokines' concentration levels between exposed and unexposed subjects, the nonparametric Wilcoxon Rank Sum Test was used. A nonparametric measure of effect size, which is a function of stochastic superiority defined as:

A = [#(Y1 > Y2) +0.5#(Y1 = Y2)]/n1n2, (Li et al., 2014), was used as A / 1 – A to give the odds that an individual in one group will score higher than an individual in the other group with respect to each cytokine. Table 3 presents these effect sizes among mothers and their babies. The cut-offs of odds A/1 – A = 1.253, 1.763, and 2.500 were used to signify small, medium, and large effect sizes respectively) (Qeadan et al., 2018; Delaney and Vargha, 2002). Graphical representations associating the effect size between cytokine concentration and level of exposure to urine U are provided. To identify polarizations in the immune response toward Th1 or Th2 responses, we applied cluster analysis using the centroid method and Ward statistics.

Finally, we used multivariate linear regression to characterize mother-to-child cytokine association (MTCA) while controlling for metal exposure. In these models using a priori scientific and epidemiological information, maternal age as a traditional risk factor and as a proxy to cumulative environmental exposures across one's lifetime was used as a predictor variable. Each model also included maternal serum cytokine concentrations predicting the same cytokines in the baby sample (e.g. maternal serum IL-2 at delivery was applied in the model to predict matched newborn cord blood IL-2 concentrations).

Log-transformed cytokine concentrations were used in multivariate linear regression models in which urinary U, and mother cytokine concentrations were applied as predictors of baby cytokine concentrations. Cytokine concentration values were also evaluated in the context of potential presence of chronic conditions (diabetes, hypertension, hypothyroidism, kidney disease etc.) observed in mothers. One baby who had low birth weight and was born premature was excluded from the final analyses ($N = 52$ babies).

The National Health and Nutritional Survey 50th percentile for urine U concentration (0.007 μg/l) for non-smoking US adults (U.S. Department of Health and Human Services Centers for Disease Control and Prevention Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, 2019) was used as an arbitrary value to characterize exposure in urinary samples as being either above or below national norms in some of the presented analyses. As there are no NHANES data for newborn baby urinary U concentrations, we utilized the NHANES 50% concentration for the youngest available group (6–11 year-old children) which is the same as NHANES adults (U.S. Department of Health and Human Services Centers for Disease Control and Prevention Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, 2019). This categorization allows us to examine trends associated with exposures above national norms with the understanding that the upper and lower toxicity levels of U are not established and that U concentrations below the arbitrary cut off may also be harmful.

3. Results

3.1. Demographics and exposure features of study participants and All NBCS mothers

Participants included in this analysis were not statistically different from the total enrolled mothers of the Navajo Birth Cohort Study (Table 1), therefore this substudy is representative of the NBCS study population overall. There were no documented occupational or hobby-related exposures to metals. Only a small portion lived close to abandoned U mines or milling sites (~ < 5%), as determined by geospatial analysis of their home location. Among participants who had below LOD urinary U concentrations the age was somewhat younger (24 yrs) resulting in a significant difference compared to women with higher urinary biomonitoring results of U ($p = 0.036$) potentially indicative of a trend toward cumulative exposures associated with age and stable, longterm residency on Navajo Nation (Erdei et al., 2019). These results are shown in Supplemental Table 1.

3.2. Uranium (U) exposure in mothers and babies

The distribution of urinary U determined in mothers and their babies, are summarized in Table 2 using the five numbers summary (minimum, 1st quartile, median, 3rd quartile, and maximum) format.

In addition, to consider lower U exposures within this sample set, the LOD value of urinary U concentration was considered as a threshold and is presented in Supplemental Table 3.

The percentage of NBCS mothers with urinary U concentrations at or above the NHANES 50th percentile of 0.007 μg/l (U.S. Department of Health and Human Services Centers for Disease Control and Prevention Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, 2019), was 71.15% (37/52) and 25% (13/52) among NBCS newborn babies (Table 3). Thirty-two percent (12/37) of mothers with U excretion above the national median had babies at birth with urinary U concentrations above 0.007, while 6.67% of mothers with urinary U concentrations lower than the median of NHANES had babies with concentration (1/15) above this cut off (McNemar's test $\chi^2 = 22.15$, df = 1, $p < 0.001$. Overall 92.31% (12/13) of babies with detectable urine U had

mothers who had urine U concentrations above the cut off. This indicates an association between U exposure of mothers and U exposure of babies at birth. Fig. 1 illustrates the overall distribution of the log-transformed U values for both mothers and babies across all concentration ranges.

A linear regression, using the logarithmic transformation on urinary U concentrations further illustrates that higher than NHANES median urinary U concentrations in NBCS mothers were associated with higher concentration of U in the babies (Fig. 2).

In fact, an increase of 10% in the level of U in mothers was statistically significantly associated with an increase of 3.37% in urinary U concentration increase in the newborn NBCS babies ($\hat{\beta} = 0.337$, $t = 2.48$, $p = 0.0166$). This association is substantial given that U levels in mothers can explain about 11% of the variability of urinary U levels in babies at birth ($R^2 = 0.1094$).

3.3. Immunological alterations

In order to characterize cytokine profiles associated with maternal or prenatal exposures to U, we assessed cytokine profiles for women who had higher than the national median concentration of urinary U versus those participants who were below the national median concentration for both mothers and babies. Univariate linear regression models were employed to examine associations of all individual cytokines (log-transformed values were used) with urinary U as a continuous variable (applied as also log-transformed variable). Urinary U positively predicted GM-CSF, and IL-2 concentrations and the association was borderline significant for IL-10 (data not shown in tables).

There were no statistically significant differences between median concentrations of cytokines for mothers with urinary U concentrations above the NHANES national median value of 0.007 μg/l and those mothers who were below the NHANES national median value for urine U. (Table 4). However, marginally statistically significant (or on the boundary of significance) differences existed when comparing babies with higher than the national median concentration of urinary U excretion and those below the median. The most pronounced differences were detected in the concentration of IL-8 for moms (increased in association with increased urine U) and decreased IFN-γ in babies who had higher than the national median concentration of urinary U excretion. Overall, effect sizes for the differences between participants with U above national norms were more evident among babies than mothers. In particular, there were seven cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-10, GM-CSF, and TNF-α), in babies for which the effect size of the difference between babies with higher versus lower than the national NHANES urinary U concentrations was medium to large (ORs of 2.21 (1.08–4.52) through 1.71(0.76–3.83). In contrast, only three cytokines (IL-8, IL-12p70, and TNF-α) in mothers' sera with higher than the national NHANES urinary U had effect sizes which almost reached medium strength (Table 4).

Decreased concentrations of IL-1β, IL-2, IL-4, IL-10, GM-CSF, IL-6 and TNF-α were observed in babies with urine U above the 50% percentile of NHANES versus those below with medium effect sizes (Table 4). Mothers with urine U above the 50% percentile of NHANES had higher mean concentrations of IFN-γ, IL-6, and IL-8 with medium effect size

for IFN-γ, in contrast to decreased concentrations for these cytokines in babies who had higher urine U (Table 4). We note that IL-7, IL-13, IL-5, and IL-12p70 did not exhibit any change in median concentration in babies related to urine U concentration.

3.4. Humoral immune response changes in association with urinary U exposure

To identify any trends suggesting directional change of polarization in the immune response, we looked at results from cluster analyses as shown in Figs. 3 and 4 for NBCS moms and babies, respectively. A trend toward Th2 cytokine polarization was observed among moms with higher urine U (right panel of Fig. 3) with IL-4, IL-5, IL-10, and IL-13 residing in one cluster that is able to explain about 77% of the variability of the data. Three other clusters were also found that were made up of (Brugge and Goble, 2002) GM-CSF; (Furlow, 2014) IL-6 and IL-8, and (Navajo Nation AUM, 2007) IL-2.

A similar pattern suggestive of a Th2 response, although less pronounced, was observed among babies with higher versus lower urine U concentrations (right panel of Fig. 4) with IL-4, IL-5, and IL-13 cytokines residing in one cluster that explained about 68.7% of the variability of the data. Among babies, two other clusters made up of (Brugge and Goble, 2002) TNF-α, IL-10, IL-2, and GM-CSF; and (Furlow, 2014) IL-8, IL-1b and IL-6 were also found to be grouped together.

3.5. Metals in mothers and their association with babies' cytokine responses

After identifying associations between mother-baby pairs, immunological changes associated with urinary U excretion, and trends toward a Th2 polarization in NBCS moms and babies, the next analytical step was to characterize cytokines representing such association and investigate whether excreted U concentrations in mothers were associated with baby immune responses. Multivariate linear regression modeling was carried out by using maternal urinary U concentrations and maternal cytokine concentrations at labor, and maternal age at enrollment as predictors for cytokine concentrations in the babies. These variables were applied after univariate linear regression models demonstrated their predictive value in such association analyses. Table 5 summarizes significant results of the multivariate linear regression modeling.

Urinary U concentrations in moms were negative predictors of GM-CSF and IL-2 concentrations in newborns. Increased maternal U exposures were found to have a negative effect on cellular stimulatory cytokines (GM-CSF works for macrophages, IL-2 on T cells) at birth. However, maternal age was a positive predictor of individual cytokine detections in case of IL-1β, and IL-2 in babies. Increased maternal serum concentrations of GM-CSF, IL-1β, and IL-2 were associated with decreased concentrations of these same cytokines in babies at birth. Furthermore, maternal serum IL-4 at delivery was significant predictor of the babies' IL-4 concentrations.

4. Discussion

Metal biomonitoring information generated for the NBCS participants showed urinary U excretion above the national median concentrations among pregnant, young Navajo females who have never been workers of the U industry (Hoover et al., 2020), as they were born after

all mining activities ceased on the Reservation. Even more notably, U contamination was also detected among newborn babies, making the Navajo Birth Cohort Study (NBCS) the first population-based birth cohort examination confirming in utero U exposures originated from low, community-level environmental pathways. About one third (32.43%) of newborn babies born to mothers have urinary U concentrations at or above the national median. While there are no national norms established for U concentrations in newborn babies, the fact that some of the NBCS newborn babies' urinary U concentrations were higher than the national average of urinary U concentration measured among NHANES non-smoking adults is a novel and concerning finding. Further, this observation substantiates concerns from the Navajo communities regarding ongoing exposures from living in proximity to abandoned uranium mines, milling sites and wastes.

Changes in median cytokine concentrations, suggestive of immune dysregulation were associated with U exposure, mainly among babies. In particular, eight out of 13 cytokines had a medium to large effect size for the difference in median concentrations in babies that had higher than the national median urinary concentration of U (0.007 μg/ml) versus that those babies with lower than the national median urinary U concentration (Table 4a and 4b). This indicates that cytokine levels in babies' cord blood are associated with maternal U exposures during pregnancy. Other studies previously indicated that perinatal factors may influence immunological cytokine responses of newborns (Szpecht et al., 2016; Lusyati et al., 2013; Gbédandé et al., 2013; Hong et al., 2015; Satar et al., 2008; Branch et al., 2010).

Although the production of Th2 cytokines was not increased among babies with elevated urinary U concentration compared to babies with low urinary U concentration, IL-4, a type of Th2 cytokines were overall increased among babies when their presence among mothers was also increased. This might be why the cluster of cytokines affected by metals in the NBCS samples showed some suggestions of polarization toward Th2 cytokines (cluster of IL-4, IL-5, IL-10, and IL-13) however, this small set of study samples does not allow conclusion of that yet. We note however that the directional change differences of cytokines (increases found in mothers, but decreases among babies) associated with U excretion underlines the complexity of the possible molecular effects that would worth further investigation among larger set of baby samples. Th2 cytokines are known to be produced in an amplified amount during normal pregnancy especially after the first trimester (Williams, 2012). This is a suggested crucial immune tolerance mechanism during pregnancy. The overproduction of Th2 cytokines can influence the Th1/Th2 cytokine balance in mothers as it is noted in the literature (Somerset et al., 2004; Tafuri et al., 1995; Raghupathy, 1997; Aghaeepour et al., 2017; Yockey et al., 2018). It is proposed by the literature that Th2-type of cytokine production supports physiological changes and the mother's body to tolerate the semi-allogenic fetus. Tolerogenic conditions are mounted as a result of downregulation of Th1-associated cellular activation and inflammatory processes (Barnes and Marsh, 1998; Li et al., 2016). However, these cytokine productions have not yet been investigated in population-based environmental health studies where exposures to metals associated with mining waste might also influence immunological as well as birth and child developmental outcomes.

Genetic changes in IL-4 and TNF-α cytokine productions of mothers and in their babies was previously investigated in other communities and ethnicities in the context of childhood asthma and allergic diseases (Barnes and Marsh, 1998; Li et al., 2016; Zhang et al., 2016), in intestinal inflammatory diseases as well in preterm babies (Schreurs et al., 2019). Altered cytokine expression, including decreased TNF-α cytokine concentrations in babies (not significant in this substudy) who had higher urine U could be related to a decreased capacity to fight common bacterial and viral infections, a concern frequently voiced by the NAIHS medical providers and by Navajo community members. Therefore, the observed cytokine alterations in this study draw attention to potential trends, but yet understudied effects of environmental U exposures in utero affecting long-term health and development in children from exposed communities.

4.1. Limitations of this analysis

Enrollment into this study could potentially be affected by participants' access to care, transportation and willingness to participate in the main parent study and produce a selection bias. However, all efforts were in place to ensure community-based awareness, education and enrollment opportunities were supported through the Navajo Nation Department of Health Community Health Representatives Program. Moreover, the availability of paired serum and urine samples and/or difficulty with newborn cord blood collection could have contributed to the samples included in this first set of analysis of U in Navajo newborns. However, as presented in Table 1, and in Supplemental Table 1 as well, participants and samples included in the present analysis are not different from the entire NBCS cohort mothers. In addition to testing for other confounding factors, we evaluated the presence of chronic diseases such as asthma and/or gestational diabetes using medical record reviews and survey information. While we recognize their potential in modifying the examined responses, the sample size did not allow controlling for them during multivariate modeling.

The cytokine changes found in multivariable models are only suggestive in this phase of our laboratory examinations. We also point out that in this smaller set of 52 mom-baby pairs we were unable to fully control for confounding effects of other predictors (such as income, education etc.) or could not fully examine the influence of unmeasured exposures routes such as dietary and soil/dust contaminations in the Navajo communities and during critical times of pregnancy.

5. Conclusions

We hypothesized NBCS mothers and newborns experiencing community-level U exposures from living in proximity to abandoned U mine and milling sites across the Navajo Nation would have observable alterations in cytokine patterns in both mothers and babies at delivery. The results showed various novel newborn cord blood cytokine responses and clustering that warrant further investigations on larger sample sizes of participants. We observed that increased maternal urinary U concentrations were predictors of negative changes in individual cytokine concentrations of GM-CSF, IL-1β, and IL-2 among their newborn babies based on multivariable linear regression modeling.

In this paper, we report that mothers with elevated urine U are more likely to have newborn babies with detectable urine U concentrations, indicative of prenatal exposure. We also observed significant changes in concentrations of numerous cytokines associated with U exposures in both moms and babies which could lead to changes in birth outcomes, child development and long-term health. The fact that newborn babies' excreted urinary U concentrations were higher than the national median concentration measured among adult, non-smoker NHANES participants is a striking finding. However, the utmost public health importance of this discovery is to ensure that community concerns about U exposure pathways on child development in newborn babies and their contributions to other chronic diseases such as high blood pressure, diabetes, kidney disease and presence of autoimmunity during childhood as well as in adulthood are going to be locally monitored, recorded and investigated prospectively. Appropriate public health policies and action plans can ensure the healthy future and well-being of Navajo babies and families for many generations to come.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclaimer

This work has not been formally reviewed by the EPA. The views expressed are solely those of the authors and do not necessarily reflect those of the Agency.

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Data availability

The authors do not have permission to share data.

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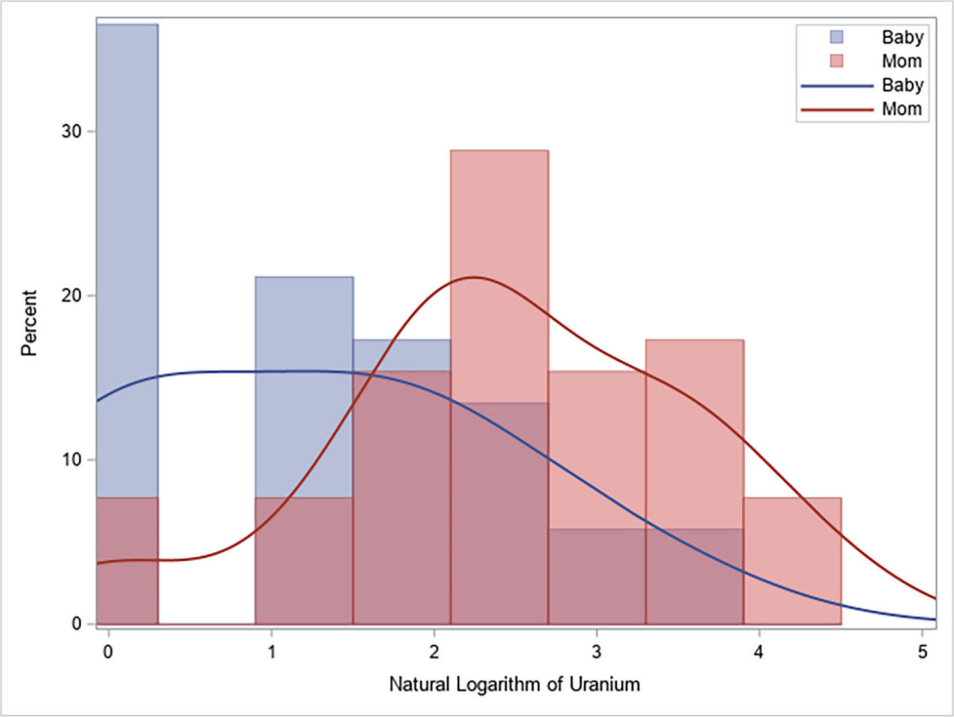

Fig. 1.

The distribution of urinary uranium (U) concentrations (after the log-transformation) for the matched pairs of mothers and babies.

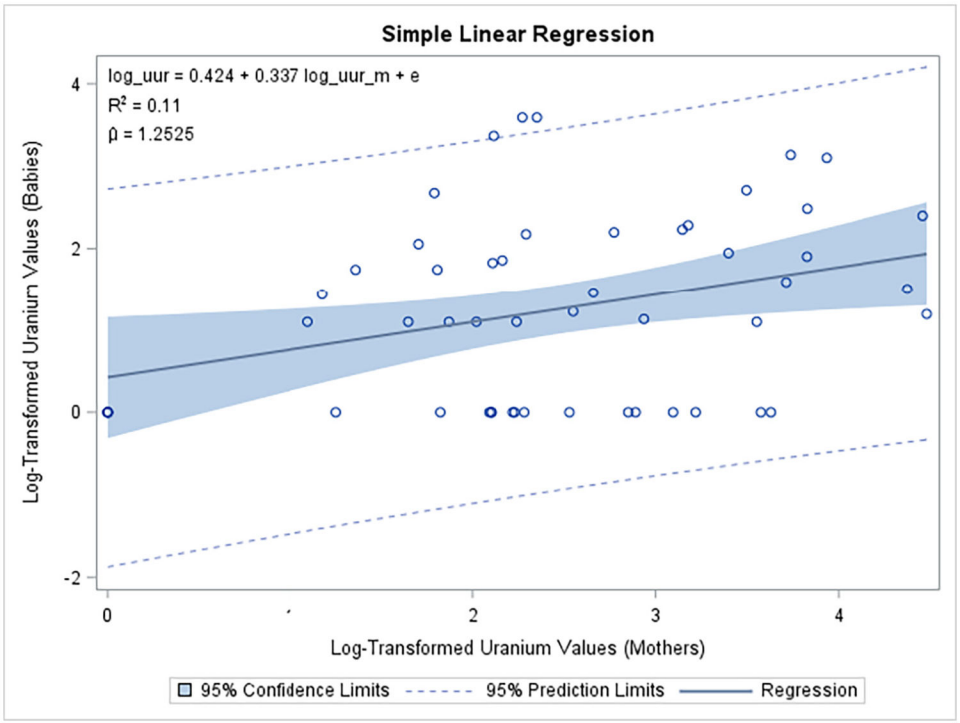

Fig. 2.

The linear association between the natural logarithm of urinary U concentration values of NBCS mothers and babies.

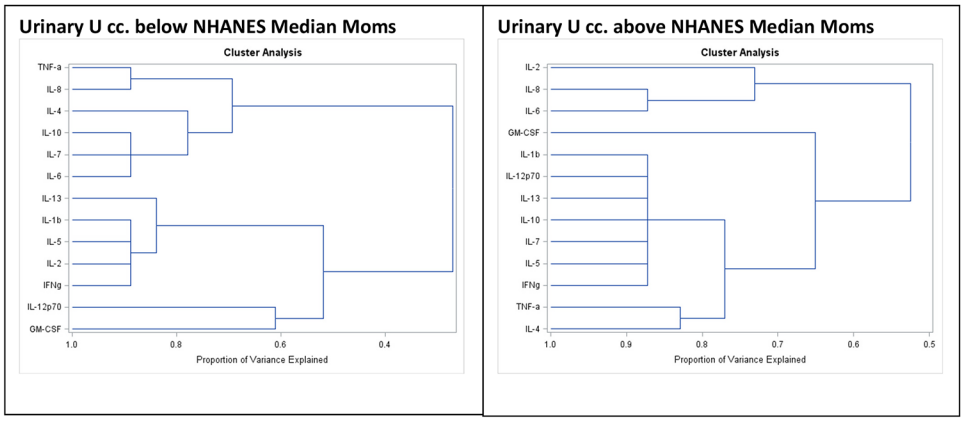

Fig. 3.

Clusters of cytokines for moms stratified by urinary U exposure status such that mothers with U excretion below the 50th percentile of NHANES adults are shown on the left panel and U excretion above the 50th percentile of NHANES adults on presented on the right panel.

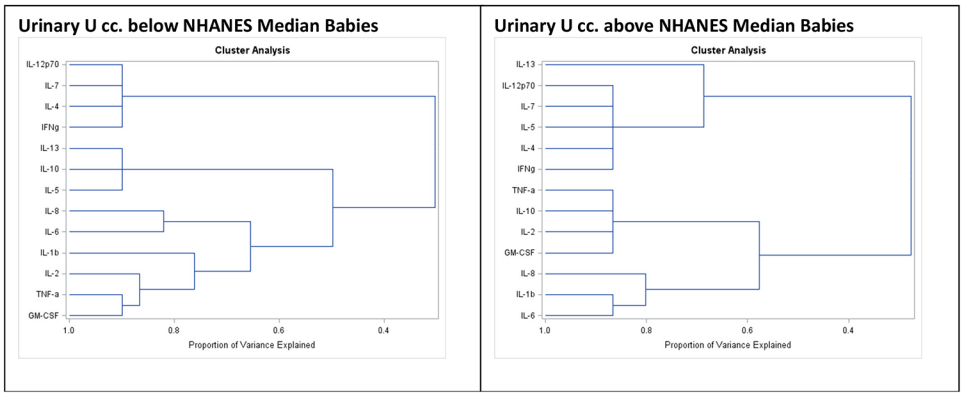

Fig. 4.

Clusters of cytokines among babies stratified by urinary U exposure status such that babies with U excretion below the 50th percentile of NHANES adults are shown on the left panel and babies' U excretion above the 50th percentile of NHANES adults are shown on presented on the right panel.

Table 1

Comparing characteristics of participants discussed in this analysis to all enrolled mothers in the NBCS.

Variable	Level	NBCS enrolled	Included in Current Analysis	p	test
N		730	52		
Age at enrollment (mean (SD), yrs)		26.76 (6.06)	26.96 (6.16)	0.818	T test
Number of current pregnancy (mean (SD))		3.09 (2.01)	3.17 (2.29)	0.787	T test
Employment status (%)	No	1 (0.2)	0 (0.0)	1.000	Fisher
	Yes	557 (99.8)	49 (100.0)		
Education – participant is currently a student (%)	No	314 (56.5)	30 (61.2)	0.806	Fisher
	Other (vocational school, dental assistant, etc.)	10 (1.8)	0 (0.0)		
	Yes	232 (41.7)	19 (38.8)		
Household Income (%)	$10,000–$19,999 per year ($834–$1666 monthly)	80 (18.5)	9 (19.6)	0.990	Fisher
	$20,000–$39,999 per year ($1667–$3333 monthly)	77 (17.8)	8 (17.4)		
	$40,000–$69,999 per year ($3334–$5833 monthly)	51 (11.8)	4 (8.7)		
	$5000–$9999 per year ($418–$833 monthly)	98 (22.7)	10 (21.7)		
	Less than or equal to $4999 per year ($417 monthly)	104 (24.1)	13 (28.3)		
	More than $70,000 per year ($5834 monthly)	22 (5.1)	2 (4.3)		
Exposed to metals through work. (%)	No	387 (95.3)	35 (97.2)	1.000	Fisher
	Yes	19 (4.7)	1 (2.8)		
Exposed to metals through hobbies (%)	No	518 (94.4)	43 (89.6)	0.198	Fisher
	Yes	31 (5.6)	5 (10.4)		
Live close to abandoned U mine site (%)	No	501 (95.1)	44 (93.6)	0.723	Fisher
	Yes	26 (4.9)	3 (6.4)		
Live close to abandoned U milling sites (%)	No	439 (98.9)	37 (97.4)	0.391	Fisher
	Yes	5 (1.1)	1 (2.6)		
Have gestational diabetes (%)	No	624 (85.8)	41 (82.0)	0.590	Chi sq
	Yes	103 (14.2)	9 (18.0)		
Have any chronic diseases diagnosed (%)	No	613 (86.0)	42 (84.0)	0.676	Fisher
	Yes	100 (14.0)	8 (16.0)		

Table 2

Distribution of urinary uranium (U) excretion concentrations among 52 Navajo mother and baby pairs.

(μg/l)	Mother (N = 52) exposures (in quantiles)					Baby (N = 52) exposures (in quantiles)				
	Minimum	25%	50%	75%	Maximum	Minimum	25%	50%	75%	Maximum
Urine U	0.0010	0.00715	0.01	0.02	0.08	0.0010	0.0026	0.00495	0.0094	0.03

Table 3

Comparison of uranium (U) exposure in 52 Navajo Birth Cohort Study (NBCS) matched pairs of mothers and babies.

	Baby's U Exposure[a]		
Mother's U Exposure[a]	**No (n) %**	**Yes (n) %**	**Total (N) %**
Below NHANES 50th Percentile (0.007 μg/l)	14	1	15
	93.33	6.67	28.85
Above NHANES 50th Percentile (0.007 μg/l)	25	12	37
	67.57	32.43	71.15
Total	39	13	52
	75.00	25.00	100.00

McNemar's test for matched pairs $\chi^2 = 22.15$, df = 1, $p < 0.001$.

[a]0.007 μg/l exposure threshold based on the 2011–2012 National Health and Nutritional Examination Survey 50th percentile for non-smoking US adults (NHANES, 2015).

Table 4

Group differences in cytokine profile by U exposure (above or below 0.007 μg/L*) among mothers (a.) and babies (b). Odds Ratios (OR and 95% CIs) = 1.253, 1.763, and 2.500 correspond to small, medium, and large effect sizes respectively.

a. Mothers

			Statistical Tests	
Variable	**Urinary U Concentrations below Median of NHANES (*n* = 15)**	**Urinary U Concentrations above Median of NHANES (*n* = 37)**	**p*a***	**OR*b***
IL-8	6.28	8.53	0.179	1.64 (0.74–4.05)
IL-12p70	6.67	3.31	0.241	1.53 (0.72–3.23)
TNF-α	8.00	6.63	0.402	1.36 (0.65–2.87)
IL-4	1.39	0.48	0.456	1.31 (0.59–2.92)
GM-CSF	460	385	0.579	1.22 (0.54–2.62)
IL-6	4.07	4.61	0.585	1.22 (0.65–2.30)
IL-10	3.26	3.16	0.606	1.21 (0.60–2.44)
IFN-γ	5.00	7.19	0.657	1.18 (0.43–1.65)
IL-2	5.06	3.03	0.960	1.02 (0.52–2.16)
IL-13	3.46	2.52	0.960	1.02 (0.53–2.02)
IL-7	8.61	7.23	0.968	1.02 (0.50–2.09)
IL-1β	1.85	1.22	0.976	1.01 (0.50–1.94)
IL-5	1.99	2.28	0.992	1.01 (0.53–1.99)

Babies

			Statistical Tests	
Variable	**Urinary U Concentrations below Median of NHANES (*n* = 39)**	**Urinary U Concentrations above Median of NHANES (*n* = 13)**	**p[1]**	**OR[2]**
IFN-γ	0.13	0.07	*0.035*	2.21 (1.08–4.52)
IL-1β	0.08	0.04	0.082	1.91 (0.88–4.15)
IL-2	0.08	0.05	0.080	1.91 (0.94–3.88)
IL-4	0.48	0.25	0.080	1.91 (0.94–3.88)
IL-10	2.76	0.24	0.113	1.83 (0.85–3.98)
GM-CSF	1.37	1.11	0.106	1.81 (0.87–3.76)
TNF-α	16.0	4.88	0.144	1.75 (0.81–3.76)
IL-6	3.74	0.08	0.160	1.71 (0.76–3.83)
IL-7	0.05	0.05	0.461	1.31 (0.72–2.38)
IL-8	18.7	15.7	0.673	1.18 (0.53–2.62)
IL-13	0.03	0.03	0.824	1.09 (0.48–1.75)
IL-5	0.06	0.06	0.903	1.05 (0.57–1.92)
IL-12p70	0.08	0.08	0.991	1.01 (0.54–1.88)

* 0.007 μg/L urinary U concentration is based on the 2011–2012 National Health and Nutritional Examination Survey 50th percentile for non-smoking US adults (NHANES, 2015). Italics represents babies' information.

[a] Wilcoxon Test.

[b] Probability-based effect size expressed as odds.

Table 5

Summary of multivariable linear regression statistical modeling results (reduced models presented) among NBCS mother-newborn baby pairs that show at least one significant predictor. Newborns' cord blood cytokines were predicted by using matched maternal cytokine concentrations (log-transformed values), maternal age and maternal urinary U concentrations. No models were rendered for log IL-12 (p70), log IL-13, log IL-5, and log IL-8. All univariate and full multivariable models were submitted and can be found in Supplemental Materials.

Cytokine response predicted in babies (log transformed, pg/mL) using above detailed predictors	**Effect estimate β**	**t- value**	***p*-value**	**Significant associations were observed**
GM-CSF	−0.106	−2.527	0.0151	logUUR mother
	−0.029	−1.639	0.1083	logGM-CSF mother
IL-1β	0.0434	1.639	0.1082	maternal age
	−0.2752	−2.482	0.0169	logIL-1β mother
IL-2	0.0163	1.441	0.1566	maternal age
	−0.1973	−3.183	0.0027	logUUR mother
	−0.1413	−3.835	0.0004	logIL-2 mother
IL-4	0.2996	3.899	0.0003	logIL-4 mother