Epidemiology of early life exposure to environmental toxicants, the microbiome, and human disease

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Outline

- In utero/early life exposure as a vulnerable window and evidence on arsenic-induced immune-related effects

- New Hampshire Birth Cohort Study
  - Potential sources of arsenic exposure during this vulnerable window
  - Emerging epidemiologic evidence on immune-related disease impacts
  - Preliminary evidence on the effects of arsenic on the microbiome
Arsenic: Class 1 carcinogen
skin, bladder and lung cancer
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CVD, PVD, lung disease, diabetes, growth, neurodevelopment, infection, mortality

Estimated 100 million people worldwide exposed to levels above the standard of 10 ug/L
Arsenic passes through the placenta
maternal-infant concentrations highly correlated

As$V$ $\leftrightarrow$ As$III$ $\leftrightarrow$ MA $\leftrightarrow$ DMA

AsB (unmetabolized)

Hall et al., 2007
In utero/early life exposures and disease risk

Smith et al., 2006; 2012; Liaw et al, 2008; Steinmaus et al., 2014;
In utero/early life exposures and disease risk

Waalkes et al., 2003; 2004; 2006; Tokar et al., 2011

Figure 1. Arsenic concentrations in Antofagasta/Mejillones water by year. An arsenic removal plant was installed in 1971.

Smith et al., 2006; 2012; Liaw et al, 2008; Steinmaus et al., 2014;
In utero/early life exposures and infection, respiratory & immune markers

- **Chile**
  - ↑ pulmonary TB \(^{Smith \ et \ al., \ 2011}\)

- **Bangladesh**
  - ↑ respiratory infection & diarrhea \(^{Rahman \ et \ al., \ 2011}\)
  - ↑ risk of wheeze \(^{Smith \ et \ al., \ 2013}\)
  - Decreased placental T cells and altered cytokines in cord blood \(^{Ahmed \ et \ al., \ 2011}\)

- **Mexico**
  - Alterations in miRNA, mRNA, proteomic markers involved in immune response \(^{Rager \ et \ al., \ 2014; \ (Baily \ et \ al., \ 2014)}\)

- **Thailand**
  - Altered gene expression in immune response pathways in cord blood \(^{Fry \ et \ al., \ 2007}\)

- **Mice**
  - delayed H1N1 clearance, and immune perturbations \(^{Kozul \ 2009}\)
Water Arsenic in USA

Karagas, 1998
New Hampshire: Mining State for Arsenic

~40% use private water systems
>10% private well As >MCL
Largely attributed to bedrock geology

Karagas, 1998
Diet as the main source of exposure

- Young children 2-3 x higher arsenic intake than adults

- Highest: fish/seafood; algae; cereal products – especially rice grains/rice-based products, bran & germ.

- Concern in US: no statutory limits for As in rice in the USA.
Diet as the main source of exposure

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“USA long grain rice had the highest mean arsenic level in the grain at 0.26 µg As g⁻¹”
New Hampshire Pregnancy Cohort

- Pregnancy
- Delivery
- Months 4-8
- Year 1-5

- 3 day diary of water, fish/seafood and rice intake
- Diet

- Private well users
- 1,500+ mother-infant pairs
- 75% response rate
- 100% urinary As
Vulnerable Exposure Windows

Maternal Gestational Exposure

Gilbert Diamond, PNAS, 2011
Vulnerable Exposure Windows

Pregnancy/In Utero

½ cup of rice/day = 1 liter of 10 ug/L As water

Early Infancy

Maternal Gestational Exposure

Gilbert Diamond, PNAS, 2011

High risk formula

Jackson, EHP, 2012

Toddler formula with organic brown rice syrup contained arsenic 6 times the EPA drinking water limit
Vulnerable Exposure Windows

Pregnancy/In Utero

Early Infancy

½ cup of rice/day = 1 liter of 10 µg/L As water

Maternal Gestational Exposure
Gilbert Diamond, PNAS, 2011

High risk formula
Jackson, EHP, 2012

Breast/Formula Feeding
Carignan, EHP, 2015

Higher exposure in formula fed infants
Rice cereal vs formula
Carignan, AGH, in press

Arsenic in 3 servings of rice cereal exceeds formula mixed with water with 10 ug/L As
Vulnerable Exposure Windows

Mid Infancy

Late infancy

Rice fed infants have higher urinary arsenic

Rice cereal vs formula
Carignan, AGH, in press

Rice and rice products
Karagas et al., JAMA Peds, in press
Vulnerable Exposure Windows

**Mid Infancy**
- Rice cereal vs formula
  - Carignan, AGH, in press

**Late Infancy**
- Rice and rice products
  - Karagas et al., JAMA Peds, in press

**Childhood**
- Rice and rice products
  - Davis et al., EHP, 2012

**Children eating rice/rice products, higher urinary As**

*Graph showing median urinary arsenic levels for Rice Eaters and Non-rice-eaters across different age groups.*

*Note: The graph illustrates the comparison of median urinary arsenic levels for children eating rice/rice products and those not eating rice/rice products, across different age groups (6 to 11 years and 12 to 17 years). The data is sourced from Karagas et al., JAMA Peds, in press, and Davis et al., EHP, 2012.*
Importance: Rice is a Staple Food

New Hampshire Birth Cohort Study

- 80% introduced to rice cereal in first year of life
- 55% eating rice or rice products, in the past two days, at age one

Karagas et al. JAMA Peds, in press
What are the health impacts of early exposure to arsenic on children?
Immune System Develops Early

- If early life exposures perturb immune-development may impact later disease occurrence
  - e.g., asthma, type-1 diabetes, inflammatory bowel disease, infections, celiac disease, juvenile arthritis and Kawasaki disease, certain childhood leukemias

- Infections primary source of childhood morbidity and mortality worldwide

- Allergies/atopies dramatically on the rise in the US and elsewhere
  - likely due to environmental agents

Dietert et al., 2010
Mechanistic & Clinical Studies

Pregnancy → Delivery → Months 4 - 8 → Year 1 - 5

Arsenic Exposure: water, urine (total & metabolites), toenails
Mechanistic & Clinical Studies

Pregnancy → Delivery → Months 4 - 8 → Year 1 - 5

Arsenic Exposure: water, urine (total & metabolites), toenails

Cord blood immune profile & epigenetics, placental gene expression
Mechanistic & Clinical Studies

Pregnancy → Delivery → Months 4 - 8 → Year 1 - 5

Arsenic Exposure: water, urine (total & metabolites), toenails

Cord blood immune profile & epigenetics, placental gene expression

Microbiome/Metabolomics
Mechanistic & Clinical Studies

Pregnancy → Delivery → Months 4 - 8 → Year 1 - 5

Arsenic Exposure: water, urine (total & metabolites), toenails

Cord blood immune profile & epigenetics, placental gene expression

Microbiome/metabolomics

Immune response measures:
Infection/Allergy/Atopy
Immune profile, vaccine response, IgE
Infant infections up to 4 months of age in relation to maternal gestational urinary arsenic concentrations (n=214)

Requiring a MD visit

RR = 1.46 (1.03, 2.07)

Requiring prescription medicine

RR = 1.60 (1.09, 2.36)

Farzan, 2013
Associations with specific infections in the first year of life

- Any Infection: 96%
- Upper Respiratory Infection: 86%
- Diarrhea: 26%
- Lower Respiratory Infection: 13%
- Wheezing: 12%

N=412

OR = 1.4 (1.1 – 1.9)
OR = 1.2 (1.0 – 1.5)
OR = 1.5 (1.0 – 2.2)

Farzan, EHP, 2015
Associations with specific infections in the first year of life

Findings for LRI, diarrhea parallel those from Bangladesh,

- OR = 1.4 (1.1 – 1.9)
- OR = 1.2 (1.0 – 1.5)
- OR = 1.5 (1.0 – 2.2)

Farzan, EHP, 2015
In utero arsenic exposure and fetal immune repertoire in a US pregnancy cohort

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Less activation could result in diminished response to vaccine, increased risk of infection

Activation by an antigen/allergen could lead to atopy

Inflammatory marker/interleukin 1 beta
Cord Blood DNA methylation arrays can be used to determine cell mixture

(1) Determined methylation profiles of leukocyte subtypes

(2) Estimated proportions of immune cells in unfractionated whole blood
   • Similar methods have been proposed for gene expression data (Gaujoux et al. 2011)
   • Allowed us to assess differences in cell type proportions based on an outcome or exposure

(3) In cord blood samples from our study, higher maternal urinary arsenic related to a greater proportion of CD8+ cells (Koestler, Marsit et al., EHP, 2013).
FIGURE 1. The developing intestinal microbiota beginning at birth. The human intestinal microbiota is shaped by environmental exposures and interacts with the developing immune system.
Arsenic & the microbiome
Hypothesize that arsenic affects the gut microbiome

- Antibiotic, immunosuppressive properties
  - Antibiotic prior to penicillin (Salvarsan, for syphilis)
  - Psoriasis medication (Fowler’s solution, potassium arsenite)

- Mouse model: 10 ppm As for 4 weeks in drinking water
  - As perturbed gut microbiome composition
  - Observed differences in metabolites

Lu K et al EHP 2014
Arsenic & the microbiome
Hypothesize the gut microbiome affects metabolism & toxicity of arsenic

In vitro human gut microbes model Van de Wiele et al EHP 2010
– Pre-systemic As metabolism is a significant process
– iAs highly methylated by colonic microbes
– Creating
  • MMA\textsubscript{v}; MMA\textsubscript{III} (highly toxic);
  • Thiolated species: MMMTAv (unknown toxicity)
Serial Analysis of the Gut and Respiratory Microbiome in Cystic Fibrosis in Infancy: Interaction between Intestinal and Respiratory Tracts and Impact of Nutritional Exposures

454 Pyrosequencing 16S rRNA (V4-V6)
• repeated respiratory and intestinal samples from birth to 21 months

• Breast feeding (gut microbiome) associated with respiratory microbiome
• Introduction of solid foods & gut microbiome

Breast feeding associated with delayed CF exacerbations, Also, increased intestinal diversity, less respiratory diversity.
New Hampshire Birth Cohort Study
Increased diversity of the gut microbiome in the 1st year of life

Diversity (SDI)

Time point

0.0
0.2
0.4
0.6
0.8
1.0

Transitional

6 weeks

1 year

p = 0.003

p = 0.15

p = 0.03

N = 149

Increased diversity of the gut microbiome in the 1st year of life

Exclusively breast fed (n=46)
Combination (n=12)
Exclusively formula fed (n=8)
Vaginal delivery (n=46)
C-section delivery (n=20)

16SrRNA, V4-V5 amplicons at MBL using Illumina MiSeq
Delivery mode and breast feeding both related to infant gut microbiome composition at 6 weeks of age

N=102

Madan, Hoen, JAMA Pediatrics, January 2016
Breast milk had very low arsenic levels.
Breast milk had **very low** arsenic levels

Breast fed infants had **low** urinary arsenic levels
Infant Arsenic at 6 weeks

Significant association between microbiome composition and
\[ \ln(\text{infant urinary arsenic concentration}) \] \( p = 0.006 \)

Among exclusively breast fed infants \( p = 0.38 \)

Among formula fed infants \( p = 0.009 \)

![Graph showing significant association between microbiome composition and infant urinary arsenic concentration.](image-url)
Maternal Gestational Arsenic

No association overall between microbiome composition and
\[ \ln(\text{maternal urinary arsenic concentration}) \] \( p = 0.45 \)

Among vaginally delivered infants \( p = 0.09 \)

Among C-section delivered infants \( p = 0.1 \)

Significant interaction between delivery mode and maternal urinary arsenic \( p = 0.02 \)
Summary of Early Life Arsenic Results

Epidemiologic data:
• ↑risk of respiratory infections; new evidence of ↑risk of wheeze

Supportive Mechanistic evidence:
• Altered cord blood lymphocyte profile, epigenetic & placental gene expression changes

Parallel findings from Bangladesh

Breast feeding
• Associated with lower arsenic
• Distinct infant gut microbiome

Preliminary arsenic-microbiome associations
• Among formula fed infants, infant arsenic exposure relates to the gut microbiome
• Maternal arsenic exposure and infant gut microbiome relationship is modified by delivery type
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