Use of Dried Blood Spots for Detection of Prenatal Alcohol Exposure

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Alcohol is a Teratogen

- Alcohol passes easily through the placenta from the mother’s bloodstream into her baby’s blood.
- The blood alcohol level (BAC) of the fetus rapidly becomes close to or equal to the BAC of the mother\(^1\).

\(^1\)Am J Obstet Gynecol 112: 387-393.
Alcohol is a Teratogen

- Ethanol elimination is impaired in the fetus due to reduced metabolic capacity and reuptake of amniotic fluid containing ethanol further prolongs fetal exposure time.

1 CDC, MMWR 61(28):534-538.
The Effect of Alcohol on Fetal Development

Periods of Fetal Development

- **Central Nervous System**: Weeks 3 to Full Term
- **Eyes**: Weeks 4½ to Full Term
- **Heart**: Weeks 3½ to 9
- **Ears**: Weeks 4¼ to 20
- **Teeth**: Weeks 6¼ to Full Term
- **Palate**: Weeks 6¼ to 16
- **Lower Limbs**: Weeks 4½ to 9
- **External Genitalia**: Weeks 7 to Full Term
- **Upper Limbs**: Weeks 4½ to 9
Fetal Alcohol Spectrum Disorders

- The neurological, behavioral and physical abnormalities that encompass the effects of prenatal alcohol exposure are collectively known as Fetal Alcohol Spectrum Disorders (FASD).

<table>
<thead>
<tr>
<th>Abnormal facial features</th>
<th>Difficulty in school (especially with math)</th>
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<tbody>
<tr>
<td>Small head size</td>
<td>Learning disabilities</td>
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<tr>
<td>Shorter-than-average height</td>
<td>Speech and language delays</td>
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<tr>
<td>Low body weight</td>
<td>Intellectual disability or low IQ</td>
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<tr>
<td>Poor coordination</td>
<td>Poor reasoning and judgment skills</td>
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<tr>
<td>Hyperactive behavior</td>
<td>Sleep and sucking problems as a baby</td>
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<tr>
<td>Difficulty with attention</td>
<td>Vision or hearing problems</td>
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<tr>
<td>Poor memory</td>
<td>Problems with the heart, kidneys, or bones</td>
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Early Diagnosis and Treatment of FASD

- While no cure exists for FASD, studies have demonstrated that early diagnosis of FASD and implementation of interventions can significantly reduce the effects of FASD and improve developmental outcomes.

- To diagnose FASD, doctors look for:
  - Abnormal facial features
  - Lower than average weight and/or height
  - Central nervous system problems including small head size, problems with attention and hyperactivity and poor coordination
  - Evidence of prenatal alcohol exposure

Detection of Prenatal Alcohol Exposure

- Maternal self-report of alcohol consumption during pregnancy
  - Prenatal records often lack information on alcohol use.
  - Maternal self-report can be unreliable due to the social stigma associated with drinking during pregnancy.
    - Alcohol Use Disorder Identification Test (AUDIT)
    - T-ACE questionnaire

- Biological markers of prenatal alcohol exposure
  - Fatty Acid Ethyl Esters in Meconium
  - Phosphatidylethanol in Blood
Phosphatidylethanol (PEth)

- Phosphatidylethanolons (PEth) are a group of phospholipids with a common phosphoethanol head group onto which 2 fatty acid chains are attached.
- PEth can form in red blood cells as a component of the cellular membrane.
- PEth is a direct alcohol biomarker, meaning that ethanol is incorporated into the final product.
When Ethanol *is not* Present

Phosphatidylcholine (PC)

Phosphatidic acid and choline

Phospholipase D (H₂O)

Red Blood Cell Membrane (Phospholipids)

Red Blood Cell
When Ethanol *is* Present

Red Blood Cell Membrane (Phospholipids)

- Ethanol

Phosphatidylcholine (PC)

Phospholipase D (Ethanol)

Phosphatidylethanol (PEth)
Detection of PEth in dried blood spots

- Our laboratory developed and validated a highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) system for the extraction and detection of PEth from DBS.

- Filter paper cards lyse and fix red blood cells and have been shown to prevent further PEth synthesis and also minimizing PEth degradation.
Degradation of PEth Over 12 Months at RT

% of Initial PEth Concentration

MONTHS STORED AT ROOM TEMPERATURE

* p < 0.001
Goals of our Newborn Screening Research

- Examine the scope of the problem of prenatal alcohol exposure in multiple populations using an objective measurement (PEth).

  Research Objective #1
  
  Is measurement of PEth from stored DBS cards from a state repository a feasible method for undertaking retrospective surveillance studies of alcohol consumption during pregnancy and rates of prenatal alcohol exposure?

  Research Objective #2
  
  Analyze rates of prenatal alcohol exposure in other “high-risk” populations where alcohol consumption during pregnancy is believed to be common.

- Establish the clinical utility of phosphatidylethanol (PEth) as a biological screening test for fetal alcohol exposure in newborns.
Research Objective #1

AIMS

1. Analyze the frequency of dried blood spot specimens that are positive for PEth.
2. Determine the prevalence rate of prenatal alcohol exposure in the general newborn population of a Midwestern State.
3. Demonstrate the efficacy of detecting PEth levels from residual, stored dried blood spot cards.

METHODS

1. Collect 250 anonymous residual blood spot specimens from the state repository via the NBSTRN Virtual Repository of Dried Blood Spots (VRDBS).
2. Elute PEth from three 3.1 mm blood spot punches in methanol, separate by HPLC, and identify and quantify level of PEth using mass spectrometry.
Concentration of PEth in Residual DBS

4% positive for PEth
8.2 ng/mL to 30.1 ng/mL
Average -17.6 ng/mL

- 10 newborns (4%) above the limit of quantitation (PEth ≥ 8 ng/mL)
- 23 newborns (9%) above the limit of detection (PEth ≥ 2 ng/mL)
- 217 newborns (87%) below the limit of detection (PEth < 2 ng/mL)
Research Objective #2

AIMS

1. Analyze the frequency of newborn dried blood spot specimens that are positive for PEth in two different populations of women considered at high risk for prenatal alcohol exposure.

   1. Women and Children’s Hospital at the Charleston Area Medical Center in Charleston, West Virginia.
   2. Neonatologist University School at the National Social Security Perinatology Unit in Montevideo, Uruguay

METHODS

1. 332 anonymous newborn dried blood cards from the CAMC Women and Children’s Hospital were examined for PEth.

2. 135 anonymous newborn blood cards from the National Social Security Perinatology Unit were examined for PEth.
The prevalence rates and levels of PEth in newborns varies significantly among different populations.

University of New Mexico Hospital
15.5% positive PEth
20 – 337.5 ng/mL
Establish the clinical utility of PEth as a biological screening test for fetal alcohol exposure in newborns.

Aim 1: Examine the prevalence and patterns of maternal alcohol consumption during pregnancy and the relationship of these reported patterns to multiple maternal biomarker levels (EtG in hair and nails and PEth in blood) in a large sample (n=1500) of pregnant women in Montevideo, Uruguay.

Aim 2: Examine correlations between maternal self-report of alcohol consumption, maternal alcohol biomarker levels and newborn PEth concentration at birth.

Aim 3: Establish and validate PEth as a newborn biomarker for prenatal alcohol exposure by establishing the sensitivity and specificity of PEth and the predictive value of newborn PEth levels.

Aim 4: To establish the half-life and window of detection of PEth in newborns by examining the kinetics of PEth elimination.

Aim 5: Analyze the relationship between maternal alcohol consumption, newborn PEth levels and developmental outcome in a subset of exposed and non-exposed infants.
Thank You!

NBSTRN
Newborn Screening Translational Research Network

Virtual Repository of Dried Blood Spots

Iowa Department of Public Health
Promoting and Protecting the Health of Iowans

State Hygienic Laboratory at The University of Iowa

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