Fatty Acid Ethyl Esters in Meconium of Infants of Diabetic Mothers: A Pilot Trial

NCT02308735

Document Approved 24 March 2017

Submitted: 22 December 2020

RESEARCH PROTOCOL OUTLINE

Title of Project: Fatty Acid Ethyl Esters in Meconium of Infants of Diabetic Mothers: a Pilot Trial

Principal Investigator: Doug Dannaway, MD, Department of Pediatrics

Co-Investigators: Marvin Williams, DO; Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine; Katherine Arnold, MD, Department of Obstetrics and Gynecology; Kimberly Ernst, MD, Department of Pediatrics, Section of Neonatal-Perinatal Medicine

Abstract

Gestational diabetes mellitus (GDM) affects as many as 14% of women in the United States. Furthermore, the number of pregnant women with pregestational diabetes mellitus (PGDM) is also increasing, mainly due to an increase in the diagnosis of non-insulin dependent diabetes mellitus. A recent study demonstrated that 1.3% of pregnancies are now complicated by PGDM and that PGDM now comprises 21% of the diabetes that complicate gestations, which represents a two fold increase since 1999. One notable side effect of diabetes is an elevation of endogenous ethanol production, which in turn may result in a rise in fetal production of fatty acid ethyl ester (FAEE). FAEE found in meconium have been utilized as a marker of prenatal ethanol exposure. Therefore, FAEE elevation could call into question maternal claims of abstinence from alcohol during pregnancy. This study seeks to determine if meconium FAEE levels in the newborns of abstinent women with various classifications of diabetes mellitus are increased when compared to non-diabetic, abstaining controls.

A. Specific Aims

Hypothesis: The levels of ethanol metabolites found in fetal meconium of teetotalling pregnant women with diabetes mellitus (both gestational and pre-gestational) will be greater than that found in teetotalling women without diabetes mellitus.

Objectives: 1) To compare meconium FAEE levels and profiles in the infants of four groups of abstinent pregnant women: women with pregestational diabetes mellitus (PGDM); women with gestational diabetes

mellitus (GDM) with only an abnormal glucose tolerance test (White's Class A1); women with GDM with fasting hyperglycemia requiring treatment with oral hypoglycemic or insulin therapy (White's Class A2); and non-diabetic controls.

B. Background and Significance

For over a decade, meconium FAEEs have been used as a biomarker of fetal ethanol exposure. FAEEs are formed by the esterification of ethanol with free fatty acids. Once formed in the fetus, FAEEs do not cross the placenta and are therefore valid markers of circulating fetal ethanol. The availability of this biomarker has allowed epidemiologic prognostication of prenatal alcohol exposure and for identification of potentially at-risk social situations. Increased meconium FAEE levels have also been correlated with poorer neurodevelopmental outcomes in children exposed to ethanol *in utero*.

While maternal consumption of ethanol is the most obvious and direct explanation of elevated FAEEs in meconium, it is not the only possible mechanism for this finding. For example, it has been noted that FAEEs occur naturally in certain olive oils; this could explain the elevated levels of meconium FAEEs found in the children born into reportedly-abstinent mothers. Also, there are medications that may include ethanol as part of their preparations. Additionally, one must remember that ethanol is a normal by-product of human intestinal microflora metabolism. While normal maternal metabolism is unlikely to raise FAEE levels to any significant degree, increased endogenous ethanol production has been noted in patients suffering from metabolic disorders, hepatitis, cirrhosis, and, most notably, diabetes mellitus.

Gestational diabetes mellitus (GDM) affects as many as 14% of women in the United States. Furthermore, the number of pregnant women with pregestational diabetes mellitus (PGDM) is also increasing, mainly due to an increase in the diagnosis of non-insulin dependent diabetes mellitus. A recent study showed that 1.3% of pregnancies are now complicated by PGDM and that PGDM now comprises 21% of the diabetes that complicate gestations, up from 10% in 1999.

It is theoretically possible that infants of diabetic mothers may have elevated levels of FAEE in their meconium despite maternal abstinence from alcohol intake. However, there is no available literature directly studying this potential phenomenon.

Also, the meconium FAEE screen is susceptible to false positive results; a newer method of detecting *in utero* ethanol exposure has been developed.

This method looks at the concentration of an ethanol by-product, phosphatidylethanol, on newborn dried blood spots (e.g., the ones taken for newborn screening).

C. Preliminary Studies/Progress Report

Dr. Dannaway is a board-certified neonatal-perinatal medicine specialist with a publication history in the area of fetal alcohol spectrum disorder. Dr. Williams is a board-certified maternal-fetal medicine specialist, and Dr. Arnold is a resident in obstetrics and gynecology. Both have an avid interest in the fetal effects of maternal diabetes.
Dr. Ernst is a board-certified neonatal-perinatal medicine specialist with a master's degree in medical informatics.

D. Research Design and Methods

This will be a pilot study, the data from which may be used in the development of a larger, multicenter study.

Researchers will approach four groups of pregnant women at 24-28 weeks when they present for routine obstetrical out-patient appointments:

1. Those with PGDM
2. Those with White's Class A1 GDM
3. Those with White's Class A2 GDM
4. Non-diabetic controls

Patients who complete their prenatal glucose screening from 24-28 weeks will be recruited once their results have been received or confirmed unless they have been designated type 1 or 2 diabetic. Patients who have type 1 or type 2 can be recruited any time after 24 weeks.

The medical records of these women will be examined to determine self-reporting of any alcohol or other drug usage while pregnant; women who report any illicit drug use (or ethanol use) while pregnant will not be eligible for this study. A routine urine drug screen will further confirm this finding. Women who have not reported alcohol use during their pregnancy will be questioned regarding medication usage while pregnant, as some medications do contain small amounts of ethanol. Women who are judged to have not consumed alcohol during their pregnancies (intentionally or incidentally) would then be included in the study.

Demographic information about the mother would also be collected (age, parity, length of pregnancy), as would the mother's most recent glycosylated hemoglobin level; additionally, a glycosylated hemoglobin

level will be drawn on our presumptive controls (to allow for covert gestational diabetes mellitus). This lab draw would be added to the mother's routine lab studies and would not require an additional venipuncture, unless no other laboratory work would be needed at the time, in which case an extra venipuncture would be necessary.

A second urine drug screen will be performed on the mother upon her admission to OUHSC for the delivery of her baby. If both screens are negative and the baby does not meet any of the exclusion criteria, the baby will be enrolled in the study.

The initial meconium from each baby of the recruited mothers will be gathered. Approximately 1 g of meconium will be collected, frozen, and evaluated for fatty acid ethyl ester analysis at the United States Drug Testing Laboratories, Inc. We will also be sending a dried blood spot either from the placenta after delivery or from the baby which will be collected at the time of the baby's scheduled newborn screen. This dried blood spot will be evaluated for phospatidylethanol, an ethanol by-product.

Upon determination of eligibility and signing of the consent form, each enrolled mother will be compensated $25. Upon confirmation of a second negative urine drug screen and successful enrollment of their baby into the study, the mother would be awarded an additional $25.

E. Statistical Methods

This is a pilot study and will not be powered to strengthen any conclusion. The four groups will be compared to each other utilizing an analysis of variance with a multiple comparisons procedure. The diabetic group and control group will be compared via t-test.

F. Gender/Minority/Pediatric Inclusion for Research

Women and minorities will not be specifically excluded from this research protocol. The recruitment forms will be printed only in English.

G. Human Participants

1. Women seen in the low-risk and high-risk obstetrical clinics at the University of Oklahoma Health Sciences Center will be eligible for this study, as will their babies.
2. We will be seeking to enlist a total of 60 infants in this study (10 in each diabetic category, and 30 non-diabetic controls). The expected age range of the mothers approached will be approximately 16 years – 45 years of age.
3. Inclusion criteria (understood to include only abstemious women):

a). Pregnant women expected to deliver between 37 and 41 weeks gestation (controls), and their babies
b). Pregnant women expected to deliver between 37 and 41 weeks gestation who have class A1 diabetes mellitus, and their babies
c). Pregnant women expected to deliver between 37 and 41 weeks gestation who have class A2 diabetes mellitus, and their babies
d). Pregnant women expected to deliver between 37 and 41 weeks gestation who were diagnosed with diabetes mellitus prior to their pregnancy, and their babies.

4. Exclusion criteria:
   a). Babies whose mothers self-reported any alcohol or any illicit drug use during their pregnancy
   b). Babies whose mothers had a positive drug screen at any point during their pregnancy
   c). Babies whose mothers suffered a placental abruption during their pregnancy.
   d). Babies whose mothers had inadequate prenatal care (defined as <3 prenatal clinic visits prior to admission for delivery)
   e). Non-English-speaking mothers
   f). Babies who pass meconium *in utero*.
   g). Babies born with multiple congenital anomalies or abdominal wall defects.
5. Each participant will be de-identified and assigned a number based on their sequence of enrollment and the particular arm of the study that their clinical status would ascribe them. This information would be kept on a password-protected database.
6. Information obtained during this pilot study would be used as the basis upon which to request funding for a larger-scale study. The study would enable the researchers to determine if maternal diabetes mellitus increases the chances of having babies who have elevated levels of FAEEs in their meconium. This information could alter the approaches that physicians and epidemiologists take toward this finding.
7. The information that could be obtained from this study far outweighs the negligible risk of accidental release of protected medical information.

H. Data and Safety Monitoring Plan

Adverse events, should they occur, will be reported to the IRB in a timely fashion.

I. Literature Cited

1. Bentley-Lewis R, Levkoff S, Stuebe A, Seely EW. Gestational diabetes mellitus: postpartum opportunities for the diagnosis and prevention of type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab. 2008 Oct; 4(10):552-8.
2. Bell R, Bailey K, Hawthorne G, Critchley J, Lewis-Barned N, on behalf of the Northern Diabetic Pregnancy Survey Steering Group. Trends in prevalence and outcomes of pregnancy in women with pre-existing type I and type II diabetes. BJOG. 2008; 115:445-452.
3. Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005. Diabetes Care. 2008 May; 31(5):899-904.
4. Pichini S, Marchei E, Vagnarelli F, Tarani L, Raimondi F, Maffucci R, Sacher B, Bisceglia M, Rapisardi G, Elicio MR, Biban P, Zuccaro P, Pacifici R, Pierantozzi A, Morini L. Assessment of prenatal exposure to ethanol by meconium analysis: results of an Italian multicenter study. Alcohol Clin Exp Res. 2012 Mar; 36(3):417-24.
5. Bearer CF, Jacobson JL, Jacobson SW, Barr D, Croxford J, Molteno CD, Viljoen DL, Marais AS, Chiodo LM, Cwik AS. Validation of a new biomarker of fetal exposure to alcohol. J Pediatr. 2003 Oct; 143(4):463-9.
6. Ostrea EM Jr, Hernandez JD, Bielawski DM, Kan JM, Leonardo GM, Abela MB, Church MW, Hannigan JH, Janisse JJ, Ager JW, Sokol RJ. Fatty acid ethyl esters in meconium: are they biomarkers of fetal alcohol exposure and effect? Alcohol Clin Exp Res. 2006 Jul; 30(7):1152-9.
7. Otasević V, Lazović V, Spalević M, Marinković O. [Endogenous alcohol in patients with diabetes and in patients with severe liver disease]. Med Glas. 1972 Nov; 26(11):391-4. Serbian
8. Simi M, Ajdukovic N, Veselinovic I, Mitrovic M, Djurendic-Brenesel M. Endogenous ethanol production in patients with diabetes mellitus as a medicolegal problem. Forensic Sci Int. 2012 Mar 10;216(1-3):97-100.
9. Peterson J, Kirchner HL, Xue W, Minnes S, Singer LT, Bearer CF. Fatty acid ethyl esters in meconium are associated with poorer neurodevelopmental outcomes to two years of age. J Pediatr. 2008 Jun;152(6):788-92.
10. Gareri J, Lynn H, Handley M, Rao C, Koren G. Prevalence of fetal ethanol exposure in a regional population-based sample by meconium analysis of fatty acid ethyl esters. Ther Drug Monit. 2008 Apr;30(2):239-45.
11. Zelner I, Shor S, Lynn H, Roukema H, Lum L, Eisinga K, Koren G. Clinical use of meconium fatty acid ethyl esters for identifying children at risk for alcohol-related disabilities: the first reported case. J Popul Ther Clin Pharmacol. 2012;19(1):e26-31.
12. Bakhireva LN, Leeman L, Savich RD, Cano S, Gutierrez H, Savage DD, Rayburn WF. The validity of phosphatidylethanol in dried blood spots

of newborns for the identification of prenatal alcohol exposure. Alcohol Clin Exp Res. 2014 Apr;38(4):1078-85.