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Validity and Reliability of Perinatal Biomarkers after Storage as Dry Blood Spots on Paper

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Abstract

Ojective—To validate use of chip-based immunoaffinity capillary electrophoresis on dry blood spot samples (DBSS) to measure obesity-related cytokines.

Methods—Chip-based immunoaffinity capillary electrophoresis was used to measure adiponectin, leptin and insulin in serum and DBSS in pregnant women, cord blood, and infant heelstick at birth and 6 weeks. Concordance of measurements was determined with Pearson's correlation.

Results—We report high concordance between results obtained from serum and DBSS with the exception of cord blood specimens.

Conclusions—Ease of sample collection and storage makes DBSS an optimal method for use in studies involving neonates and young children.

Keywords

dry blood spot; adionectin; leptin; insulin

Introduction

Adiponectin, leptin and insulin are important cytokines in the study of obesity and the metabolic syndrome. These measurements may be of value in studies of the interaction between an infant's genotype and intrauterine environment and subsequent risk of adult obesity and metabolic syndrome. Studies of these relationships have been hampered by the difficulties of collecting adequate volumes of serum from newborn infants. As a result, most measure cytokine concentrations in cord blood.(Chiesa and others, 2008; Hibino and others, 2009; Martos-Moreno and others, 2009; Yoshida and others, 2009) Only a few obtained venous or heelstick samples.(Kamoda and others, 2004; Kyriakakou and others, 2008; Mazaki-Tovi and others, 2007)

Mandated newborn screening for a variety of genetic disorders utilizes dry blood spots that are routinely collected shortly after birth. Dry blood spots also have been used with enzyme

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immunoassays to measure other bio-markers, including neurocysticercosis, leptin, and human transferrin receptor.(Fleury and others, 2001; McDade and Shell-Duncan, 2002; Miller and others, 2006) These studies have reported good concordance and high sensitivity and specificity between the dry blood spot measurements and serum measurements. One study used dry blood spot samples (DBSS) to measure adiponectin in small and appropriate for gestational age infants using sandwich immunoassay and demonstrated correlation of r=0.87 between DBSS and serum measurements.(Klamer and others, 2007) Traditional enzyme immunoassays for adiponectin, leptin and insulin require cellular volumes of 50 microliters or more.(Miller and others, 2006) This report describes the results of a validation study to compare levels of adiponectin, leptin and insulin in DBSS compared with serum samples using a chip-based immunoaffinity capillary electrophoresis method capable of analyzing very small sample sizes (less than 1 microliter) in about 60 minutes.(Phillips, 2004)

Methods

Following approval by the Institutional Review Board at the University of Utah, we recruited patients from the obstetrics clinics at the University of Utah between September 2006 and December 2007. The purpose of the study was explained to potential participants who were at 20-34 weeks gestation based on post-menstrual age (PMA). Those who agreed to participate signed consent forms at time of enrollment. All participants had singleton pregnancies confirmed on 2-D ultrasound at 20 weeks. Parental permission for infant studies was obtained after birth.

Samples consisted of DBSS and capillary blood for serum analysis. Specimens were collected at four timepoints: 1) maternal fingerstick at 35 weeks PMA; 2) mixed venous and arterial cord blood at birth; 3) infant heel stick within 48 hours of birth; and 4) infant heel stick at 6 weeks of age. Within 24 hours of collection, serum was isolated by centrifugation then placed at -80C for frozen storage until analysis. DBSS were stored at room temperature for as long as 6 months prior to analysis.

Serum concentrations of adiponectin, leptin and insulin and their reactive antibodies were measured by preparing standards of 10, 50, 100 and 500 pg/mL using an antibody-based capillary electrophoresis coupled with immunological extraction or immunoaffinity capillary electrophoresis system equipped with an on-line laser-induced fluorescence detector capable of measuring intracellular proteins in cultures as low as 100 cells.(Phillips, 2004) DBSS were reconstituted to stock solutions of 1microgram/mL in 0.1 M phosphate buffer, pH 7.4 and then analyzed using the antibody-based capillary electrophoresis system described for serum samples. Precision and accuracy of this system is capable of detecting a concentration of approximately 0.5pg. Calibration curves were constructed and analyte concentrations were calculated by comparing the area under the curve for each sample to the standard calibration curves.

Statistical analysis

Pearson's correlation was used to evaluate concordance between serum and DBSS measurements at each time point. Significance was achieved at alpha = 0.05. All analyses were performed using SPSS v17.0. (SPSS Inc., Chicago, IL)

Results

We obtained fingerstick samples from 54 women at 35 weeks PMA and 44 cord blood specimens at the time of birth. Heelstick specimens were obtained from 45 newborns within 48 hours of delivery and from 31 infants at 6 weeks of age. There was excellent agreement

for leptin and insulin concentrations obtained from pregnant women and from heelstick specimens at 48 hours and at 6 weeks. The correlation for adiponectin was excellent in maternal specimens and at 48 hours; at 6 weeks the correlation was less strong but still significant. Cord blood serum and blood spot samples showed excellent correlation for insulin, but poor correlations for adiponectin and leptin (Table).

Discussion

The results of our study, showing that there is excellent agreement between the levels of adiponectin, leptin and insulin determined from DBSS and those from serum, validate the use of the former method for studies of the significance of variations in these levels in newborn infants. This method minimizes the volume of blood to measure cytokines and eliminates the need for venipuncture. In addition, we found that the concordance between serum samples and blood spot samples that had been stored for up to six months remained high. Our findings indicate that stability over time is similar for DBSS and frozen serum and validate this mode of blood storage and collection.

The lack of correlation of adiponectin and leptin between DBSS and serum obtained from cord blood may be related to inadequate mixing of venous and arterial cord blood specimens or delayed preparation of DBSS. Miller et al reported lower stability of leptin, attributing the lower stability to inadequate refrigeration or freezing of the sample.(Miller and others, 2006) Klamer et al (2007), using multiplex sandwich immunoassay xMAP technology, reported decrease in measurable adiponectin in DBSS stored longer than 5 days at room temperature. Our findings demonstrate stability of adiponectin, leptin and insulin in DBSS stored at room temperature for as long as 6 months.

Collecting DBSS from heel stick samples of newborns is a convenient and simple method of obtaining blood samples. We conclude that they provide accurate results of the levels of adiponectin, leptin and insulin using chip-based immunoaffinity capillary electrophoresis. Scientists interested in investigating the significance of newborn cytokines can be confident that this method provides valid results. We advise caution in the use of cord blood samples for this purpose.

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Table

Pearson's correlation (R) of cytokines measured in serum and dry blood spots

	Adiponectin	Leptin	Insulin
Maternal fingerstick, 35wks PMA	0.916 *	0.962 *	0.975 *
Cord blood	0.236	−0.03	0.985 *
Heelstick, within 48 hours of birth	0.931 *	0.975 *	0.93 *
Heelstick, 6 weeks of life	0.772 *	0.937 *	0.925 *

* $p<0.0001$