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Reduced early dried blood spot citrulline levels in preterm infants with meconium obstruction of prematurity

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ABSTRACT

Background: Citrulline is a non-protein amino acid synthesized in the enterocytes of the small bowel. Recent studies have reported that plasma citrulline levels correlate with functional enterocyte mass.
Aims: This study aimed to determine the normal dried blood spot (DBS) citrulline levels and to determine the existence of a correlation between citrulline levels and meconium obstruction of prematurity (MOP).
Study design and subjects: A retrospective cohort study was performed involving 285 infants born at less than 32 weeks gestation who were admitted to the neonatal intensive care unit between Oct 2009 and Aug 2014.
Outcome measures: We analyzed the DBS citrulline levels, which are routinely measured via newborn screening at 7 days following birth, using liquid chromatography-MS/MS. We investigated the relationship between DBS citrulline levels and clinical parameters such as gestational age (GA), body measurements at birth, gender, or the presence or absence of either necrotizing enterocolitis or MOP.
Results: A total of 229 infants with a median GA of 29.6 weeks and a median birth weight of 1160 g were included. DBS citrulline levels were not associated with GA, body measurements at birth or gender. DBS citrulline levels were significantly decreased when patients presented with MOP ($p = 0.037$).
Conclusions: Early DBS citrulline levels were not associated with either GA or body measurements at birth but were reduced among preterm infants with MOP compared with the control infants. These results may be indicative of abnormal fetal intestinal development and reduced functional enterocyte mass among preterm infants with MOP.

1. Introduction

Meconium obstruction of prematurity (MOP) is an obstruction of the terminal ileum or the colon and is caused by inspissated meconium. Clatworthy et al. [1] first described meconium obstruction (MO) in 1956. The increased survival of preterm infants has resulted in an increased incidence of MOP [2,3]. The pathophysiology of MOP is poorly understood and appears to be multifactorial. Delayed passage of meconium might be because of the reduced water content and increased viscosity of meconium [4]. Additionally, delayed maturity of the interstitial cells of Cajal has been suggested as a risk factor for distal ileal MO [5].

Citrulline is a nonessential amino acid synthesized in the liver and the small intestine. As hepatic citrulline is catabolized in the intrahepatic urea cycle, intestinal production within enterocytes serves as the primary source of systemically circulating citrulline [6,7]. Many studies have suggested that citrulline is a reliable index of functional enterocyte mass [8–10]. Clinically, the plasma citrulline concentrations are reported to be significantly lower both among patients suffering from villous atrophy, short bowel syndrome, Crohn's disease, and damage following radiation exposure and following small bowel transplantation [11,12]. Several studies have postulated that the plasma citrulline levels are decreased in preterm infants with necrotizing enterocolitis (NEC) compared with age-matched controls [13,14]. The relationship between citrulline and MOP has not been explored. Based on the current evidence, it has been hypothesized that early citrulline levels are abnormal in preterm infants with MOP.

The aim of our study was to determine the normal dried blood spot (DBS) citrulline levels in premature infants and determine whether a correlation exists between DBS citrulline levels and MOP in preterm infants.

2. Methods

2.1. Patients

This retrospective cohort study included infants <32 week gestation, who were admitted to the neonatal intensive care unit (NICU) of Seoul

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National University Children's Hospital between Oct 2009 and Aug 2014. We collected data regarding the gestational age, birth weight, head circumference and length, gender, and amount of enteral feedings at the time of sampling. Additionally, the clinical characteristics and outcomes, including the days to full enteral feeding and the presence or absence of NEC or MOP, were collected. The exclusion criteria included major congenital anomalies such as intestinal anomalies, metabolic disorders, and outborn infants. This study was approved by Seoul National University Institutional Review Board.

2.2. Diagnosis of MOP

All of the infants with MOP initially presented with abdominal distension, bilious gastric residue and feeding intolerance. MOP was suspected if the infants had difficulty with meconium passage and exhibited dilated bowel loops on plain radiography [15]. Abdominal ultrasonography was performed in all the patients suspected of having MOP. Ultrasonography findings included meconium-filled dilated bowel loops without evidence of NEC. In medically treated cases, the final diagnosis was confirmed via a contrast enema resulting in the passage of meconium and the resolution of feeding intolerance. In surgically treated cases, the definitive diagnosis was made via operative findings: inspissated pellets of meconium in the distal ileum or colon with distended proximal bowel, or meconium-stained ascites in case of perforation. There was no intestinal malrotation, atresia, or mesenteric defect.

2.3. Sample collection and citrulline assay

DBS samples are routinely collected for a newborn screening test at 7 days following birth. Capillary blood was obtained via heel pricks and dried on filter paper. The DBS samples were analyzed by liquid chromatography-MS/MS (LC-MS/MS). The analysis was performed with Waters Alliance 2795 (Waters, Watford, UK) and Quattro Micro tandem mass spectrometers (Waters, Manchester, UK) using mobile phase with 80% acetonitrile in water. The gradient profile was 160 μL/min during 0.24 min, 20 μL/min during 1.2 min and 180 μL/min during 1.8 min. A Quattro Micro tandem mass spectrometer was used with the following settings: capillary voltage, 3.6 kV; cone voltage, 20 V; and collision energy, 15 V.

2.4. Statistical analyses

The data are expressed as the means ± standard deviations. The non-normally distributed data were represented as a median with ranges. The relationship between the DBS citrulline levels and variables such as the gestational age, birth weight and the amount of enteral feedings at blood sampling were analyzed using Spearman's rank correlation coefficient (rs). A univariate analysis was performed to assess the differences in the DBS citrulline levels between the group with MOP and the group without MOP, using the Wilcoxon rank-sum test. The differences with a $p < 0.05$, based on the results of the univariate analysis, were entered in a multivariate logistic regression analysis. Statistical significance was set at $p < 0.05$. The statistical analysis was performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Table 1
Clinical characteristics of patient.

	MOP patients (n = 17)	Controls (n = 212)	P value
Gestational age, weeks	28.49 ± 2.35	29.24 ± 2	0.223[a]
Birth weight, g	838.82 ± 236.08	1189.11 ± 328.73	<0.001[b]
HC, cm	23.62 ± 1.45	26.31 ± 2.37	<0.001[b]
Length, cm	31.94 ± 3.14	37.12 ± 3.68	<0.001[a]
SGA	6 (35.29%)	23 (11.92%)	0.017[c]
Male:female ratio	1.1:1	0.7:1	0.648[d]
Enteral feeding (mL/kg/day)	6.7 ± 15.3	73.4 ± 60.1	<0..01[a]

HC = head circumference, SGA = small for gestational age.
[a] Wilcoxon rank sum test.
[b] Independent *t* test.
[c] Fisher's exact test.
[d] Chi-square test.

3. Results

3.1. Patient clinical characteristics

A total of 285 preterm infants were admitted during the study period. We excluded 37 infants, including 24, 5 and 8 patients because of incomplete data, major congenital anomalies, and death within 48 h following birth, respectively. We enrolled 248 preterm infants, including 17 with MOP and 231 without MOP. Thirteen infants with stage ≥ 2 NEC and 6 with spontaneous intestinal perforation were subsequently excluded from the "No MOP" group because of the possibility of impaired intestinal function. Therefore, 17 preterm infants with MOP and 212 without MOP constituted the MOP group and the control group, respectively (Fig. 1). Table 1 includes the demographic characteristics of the infants with MOP and those of the control infants. The gestational age was comparable between the groups. Body measurements such as weight, head circumference and length at birth were significantly lighter or smaller in the MOP group. The amounts of enteral feeding differed significantly between the MOP group and the control group at the time of the DBS blood sampling.

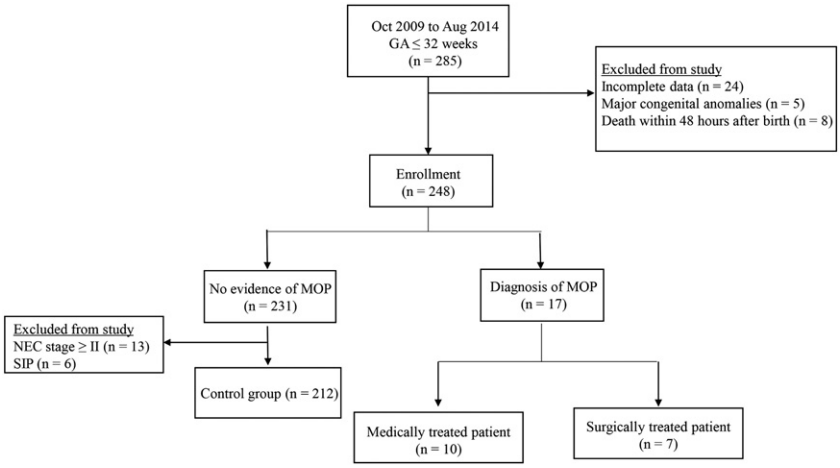

Fig. 1. A flow chart depicting study enrollment and meconium obstruction of prematurity in preterm infants.