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RESEARCH ARTICLE

Is Meconium Stained Amniotic Fluid Related to Cord Blood Vitamin D Levels?

Saynur YILMAZ[1,2], [MD]
Funda AKPINAR[1], [MD]
Ayla AKTULAY[2], [MD]
Ozlem Gun ERYILMAZ[2], [MD]
Zehra Ozturk BAŞARIR[1], [MD]
Canan DEMIRTAS[3], [MD]
Yaprak ENGIN-USTUN[2], [MD]
Leyla MOLLAMAHMUTOGLU[1], [MD]

1 Etlik Zubeyde Hanim Women's Health Training and Research Hospital
2 Zekai Tahir Burak Women's Health Training and Research Hospital
3 Gazi University Faculty of Medicine, Department of Biochemistry

* *Corresponding Author: Saynur Yilmaz, MD, Etlik Zubeyde Hanim Women's Health Training and Research Hospital Ankara, Turkey*
e-mail: saynur77@yahoo.com

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ABSTRACT

Aim: The present study aimed to compare cord blood Vitamin D levels of infants born with meconium stained amniotic fluid to those with clear amniotic fluid.

Method: Term pregnant women in the active phase of labor and having meconium stained amniotic fluid were defined as the study group (n=44). Women with healthy uncomplicated pregnancies with clear amniotic fluid matched with the study group for age, parity and gestational age were defined as the control group. Demographic, delivery and laboratory parameters of both groups were recorded. Cord blood vitamin D levels were measured with high performance liquid chromatography.

Results: The 88 women were between the ages of 18-40 years. There were 16 patients with massive meconium, and 28 patients with mild and moderate meconium. Mean vitamin D levels of the study and control groups were 45.1 and 41.0 ng/ml, respectively. In our study sample, 14% of the study group and 23% of the control group had deficient levels of vitamin D (≤20 ng/ml 25(OH)D). Vitamin D levels did not correlate with the presence of meconium or the thickness of the meconium.

Conclusion: The present study indicates that blood levels of vitamin D of infants born through meconium stained amniotic fluid were not significantly different from those with clear amniotic fluid. Larger studies are needed to define the exact role of vitamin D in pregnancy and to investigate the effect of additional Vitamin D intake on pregnancy outcomes.

Key words: Meconium, cord blood, vitamin D, low risk pregnancies.

Introduction

Meconium-stained amniotic fluid (MSAF), is defined as the passage of fetal intestinal lumen content into the amniotic cavity. It is a frequent situation in obstetric practice with an incidence of 12% of all deliveries [1,2]. Many authors agree that meconium passage occurs as a result of fetal hypoxia and placental dysfunction [3,4]. Thick meconium has been found to be the most important factor effecting fetal outcome [3].

The classical functions of vitamin D are related to bone mineral metabolism. But recent studies have shown that vitamin D receptors are expressed in almost all cells of the body, suggesting that vitamin D has many more functions than already known [5]. In pregnancy, Vitamin D plays autocrine, paracrine, and endocrine roles in trophoblast invasion, nutrient and gas exchange [6,7]. Vitamin D deficiency in pregnancy is reported to be related with preeclampsia, gestational diabetes mellitus, preterm birth and small-for-gestational age babies [8]. Shemer and Marschall evaluated vitamin D levels of women with intrahepatic cholestasis of pregnancy [9]. They found lower vitamin D levels in meconium stained deliveries.

Due to the fact that the risk of adverse pregnancy outcomes increases in vitamin D deficient mothers and that meconium stained amniotic fluid has been reported as a sign of placental dysfunction, we hypothesized that vitamin D might have a role in the development of the pathology of meconium stained amniotic fluid.

The aim of this study was to investigate whether cord blood vitamin D levels are different between meconium stained and unstained amniotic fluid.

Materials and Methods

This study was carried out at the Zekai Tahir Burak Maternity and Women's Health Training and Research Hospital between January and March 2013. Forty-four women with singleton pregnancies complicated by MSAF between 37 and 41 weeks of gestation were defined as the study group and these patients were matched with 44 healthy uncomplicated pregnancies with clear amniotic fluid for age, parity, and gestational age who were defined as the control group. Patients both in the study group and the control group were non-smokers and were taking multivitamin tablets daily. Patients with multiple pregnancies, maternal systemic disease, preterm birth, intrauterine growth retardation, fetal anomaly, small for gestational age, preeclampsia or gestational diabetes mellitus were excluded from the study. This study was approved by the local ethics committee (No: 24/July 18, 2012) and performed in accordance with the ethical standards for human research established by the Declaration of Helsinki. Informed consent was obtained from all participants.

Gestational age was determined based on crown rump length measurement by ultrasonography performed before 14 weeks of gestation. All newborns were evaluated for birth weight, gender, mode of delivery and need for neonatal intensive care unit admission. Amniotic fluid color was assessed at the time of membrane rupture and delivery. Meconium was categorized as thick, moderate or thin, based on its naked-eye appearance.

The groups were compared according to cord blood vitamin D levels. The umbilical cord was clamped and 10 ml of arterial blood was collected at delivery. Afterwards, cord blood was centrifuged at 4000*g* for 10 minutes, serum samples were stored at –80°C and analyzed at the end of the study period. Circulating vitamin D levels were assessed by measuring 25-hydroxy-vitamin D3 (25(OH)D3) in serum by high performance liquid chromatography using a kit marketed by ImmuChrom GmbH (Germany). The intra-assay coefficient of variation for serum vitamin D was 2.6% and the inter-assay coefficient of variation for serum Vitamin D was 4%. The reference interval was 25-150 nmol/l (20-60 ng/ml) for winter time.

Data were analyzed with the SPSS software version 15.0 for Windows (SPSS Inc., Chicago, Illinois,USA). Groups were controlled in terms of conformity to normal distribution (graphical check and Kolmogorov-Smirnov test). Median (IQR) was used for groups that were not distributed normally. Chi-square tests were conducted to test the distribution between categorical variables. The Mann–Whitney test was performed for not normally distributed variables and Student's t test was used for normally distributed variables. A p-value <0.05 was considered significant.

Results

A total of 88 pregnant women were included in the study: forty-four women in the MSAF group and 44 women in the control group. The groups were comparable in terms of maternal age, gravidity, parity, body mass index and mode of delivery (Table).

Fetal characteristics including birth weight and newborn gender were similar between groups (p= 0.362 and p= 0.831, respectively). One infant in the study group required intensive care unit hospitalization (p=0.545). The mean cord blood 25(OH) D3 levels of the study and control groups were 45.1 ng/ml and 41.0 ng/ml, respectively (p= 0.545). 14% of the study and 23% of the control groups were deficient of 25(OH) D3 with a level less than 20 ng/ml.

The appearance of the meconium was distributed as mild (n=7; 8%), moderate (n=21; 21%) and severe (n= 16; 18%). Mean 25(OH) D3 levels for mild, moderate and severe MSAF groups were 47.5 ng/ml, 41.64 ng/ml and 51.81 ng/ml, respectively. There was no difference between vitamin D levels of the cord blood in the three groups formed according to the thickness of meconium (p= 0.597). The cord blood vitamin D levels was similar between male and female babies (p= 0.831).

Discussion

This case control study was undertaken to compare vitamin D levels in cord blood of term newborn infants with either meconium stained or clear amniotic fluid. To the best of our knowledge, very little is known about the possible association between MSAF and vitamin D. [9]. The results of the study showed no relation between intrauterine meconium passage and cord blood vitamin D levels.

There are several studies indicating an association between low levels of Vitamin D during pregnancy and adverse pregnancy outcomes, including preeclampsia, diabetes mellitus, preterm birth and the newborn being small for gestational age [8].

Table: Demographic and clinical characteristics of the groups

Variables	Meconium group (n= 44)	Control group (n= 44)	P value
Age (years) (mean)	26.14 (4.62)	25.84 (4.53)	0.763
Gravidity (median)	2 (1-7)	2 (1-5)	0.612
Parity (median)	0 (0-4)	1 (0-2)	0.735
BMI (kg/m^2)	28.8 (4.63)	27.9 (4.27)	0.331
Gestational age (days) (mean)	276.7 (9.7)	274.9 (4.27)	0.301
Mode of delivery n(%)			
Vaginal birth	22/44 (50%)	22/44 (50%)	1.000
Cesarean section	22/44 (50%)	22/44 (50%)	
Birth weight (grams) (mean)	3368 (416)	3290 (392)	0.362
Newborn gender n(%)			
Male	25/44 (57%)	24/44 (55%)	0.831
Female	19/44 (43%)	20/44 (45%)	
NICU admission n(%)	1/44 (2%)	0/44 (0%)	0.317
Cord blood vitamin D level (ng/ml) (mean)	45.1 (33.2)	41.0 (28.7)	0.545

Mean (SD), Median (min-max), n (%)
BMI: Body mass index, NICU: neonatal intensive care unit

In preeclampsia, there is an abnormal endothelial function and also an inappropriate immune response of the mother to the fetus. The active form of vitamin D is reported to regulate the transcription of the genes associated with the placental invasion, normal implantation and angiogenesis. Vitamin D is also shown to function in immune modulation [10], which is pathogenic in both preeclampsia (between mother and fetus) and diabetes mellitus. In diabetes mellitus pancreatic β cells are destroyed by autoimmunity [11]. The relation between vitamin D and newborns small for gestational age is again based on inadequate implantation and placentation [12]. If we consider meconium passage as a result of fetal hypoxia, this might be due to the abnormal placentation and angiogenesis.

In our study, mode of delivery was comparable between MSAF and clear amniotic fluid groups. Recently association of maternal vitamin D and adverse labor and delivery outcomes was investigated [13,14]. In the study by Gernand et al. 25(OH) D concentrations were reported not to be associated with risk of prolonged stage 1 or 2, cesarean delivery or instrumental delivery [13]. While Scholl et al. reported a two fold increase in cesarean delivery rate in vitamin D deficient mothers [14]. The hypothesis behind the association of prolonged labor and increased cesarean delivery is decreased muscle power and pushing force of mother with vitamin D deficiency and associated pelvic floor dysfunction. However, since pelvic floor dysfunction is a multifactorial phenomenon, the results are inconsistent.

Vitamin D has been extensively investigated in the adult population. Various cut-offs have been used to report circulating vitamin D status. Many experts suggest that the patient should be considered to be deficient in vitamin D if the serum level of 25(OH) D is less than 20 ng/ml [15]. On the basis of this definition about 14% of the meconium group and 23% of our control group would be classified as being deficient in vitamin D. We found no significant relationship between Vitamin D deficiency and MSAF. The logical explanation behind this result could be due to the maternal vitamin D level as the mother provides calcium to fetus without a precondition of vitamin D, calcitriol, or the vitamin D receptor [8]. Our study population had higher cord blood vitamin D levels compared to some studies [16,17]. We used HPLC method which is the reference method for measuring vitamin D levels. Discrepancy between cord blood vitamin D levels could be attributed to the differences in methods of measurement and the kits

used. The disparity between vitamin D levels in an earlier study conducted with pregnant women and their newborns in Turkey [18] and our study might be due to the subjects being from different parts of the country and seasonal difference.

There are also several reports that have evaluated the difference in vitamin D levels according to gender in adults. They demonstrated higher levels of vitamin D in males compared to females. The authors related this result to the amount of time spent outdoors, or clothing differences [19,20]. Less is known about the relationship between vitamin D and sex during fetal development [21]. When we evaluated cord blood vitamin D levels according to fetal sex we found no difference. This was a secondary outcome of our study. Given the interrelation of sex steroids with vitamin D [22], this subject should be evaluated in larger series.

Potential limitations of our report include the following. Firstly, we measured Vitamin D levels in cord blood in a small number of participants. Secondly, multiple measurements throughout the pregnancy may have demonstrated more reliable results. Thirdly, although this was a prospective study, we did not record the signs for duration of intrauterine meconium exposure such as meconium staining of the vernix, umbilical cord and nails. Strength included subjects from multiple geographical areas of the country.

Although vitamin D supplementation during pregnancy is advised for better maternal and fetal outcome, we did not find a correlation between cord blood 25(OH) D concentrations with meconium staining or the thickness of meconium staining.

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