Original Article

DOI: 10.4274/haseki.galenos.2024.9397
Med Bull Haseki 2024;62:22-28

Comparison of Vitamin D and Calcium Levels Between Hospitalized Refugee Newborns and Native Newborns with Early-onset Hypocalcemia

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Abstract

Aim: Vitamin D deficiency (VDD) is mainly listed in the etiology of late-onset hypocalcemia in the neonatal period, as a probable cause of hypocalcemia in the early period, figured out in our study. We compared the vitamin D status and biochemical characteristics of refugee newborns with those of native newborns with early-onset hypocalcemia.

Methods: One hundred and forty newborns enrolled in our comparative cross- sectional study were admitted with calcium <8 mg/dL in term or <7 mg/dL in preterm infants detected at the maternity ward within 72 h postnatal age during a 3-month period from June to August in 2020. Serum calcium, phosphorus, magnesium, alkaline phosphatase, and parathormone levels were measured on the initial day. Vitamin D and calcium levels in newborns before discharge.

Results: The serum calcium levels in refugees were 7.12 mg/dL and 7.23 mg/dL in native newborns. The median vitamin D level was 8.57 µg/L in refugees and 7.99 µg/L in native newborns. Vitamin D deficiency was found in every eight in ten newborns with early-onset hypocalcemia. There was no difference in the prevalence of VDD between the refugee and native neonates. Maternal vitamin D supplementation was 12.8% in the refugee group and 13.3% in the native group. The hospital stay was similar in both groups.

Conclusion: Adequate access to preventive health services, routine screening of pregnant women for VDD, and supplementation during pregnancy and lactation should be provided to mothers from underdeveloped or developing countries because the prevalence of VDD is higher among this group.

Keywords: Hypocalcemia, newborn, refugee, vitamin D

Introduction

Calcium plays a role in several metabolic processes and bone mineralization (1,2). Hypocalcemia is one of the most common electrolyte imbalances during the neonatal period, is seen within the first three postnatal days, and is accepted as early-onset hypocalcemia (3). Causes of early-onset neonatal hypocalcemia are intrauterine growth restriction, prematurity, perinatal stress/asphyxia, being an infant of a diabetic or preeclamptic mother, septicemia, maternal history of antiepileptic use, and hyperparathyroidism (4). Although early-onset hypocalcemia is not primarily symptomatic, apnea, tremor, cyanosis, poor feeding, vomiting, and focal and generalized seizures are the main clinical symptoms of hypocalcemia (5). Treatment for newborns with any clinical symptom of hypocalcemia should be given for at least 3 days. The majority of infants with early-onset hypocalcemia improve after 48-72 hours of treatment in most cases, hopefully without any significant complications (6).

Vitamin D levels in newborns mainly depend on maternal vitamin D status, breastfeeding, and sunlight exposure (7,8). Causative factors for maternal vitamin D deficiency (VDD) include darker skin pigmentation, skin coverage with whole-body clothing, which directly affects neonatal vitamin D status, and residing at high altitude. Vitamin D deficiency results in hypocalcemia, hyperphosphatemia,

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Received: 07.08.2023 **Accepted:** 23.02.2024

and elevated levels of alkaline phosphatase (ALP) and parathormone (PTH) as calcium homeostasis is disrupted. In some cases, only hypocalcemia can be a manifestation of VDD.

The civil war in Syria resulted in an enormous refugee crisis, and Turkey hosts approximately 4 million Syrian refugees as the largest refugee group in the country (9). The well-being of refugee infants has been negatively affected because of the poor nutrition of their mothers. Inadequate access to preventive healthcare services also causes short- and long-term undesirable complications for both infants and mothers. Implementation of a properly organized health support program plays a crucial role in raising awareness about preventive strategies via vaccination and routine screening of mothers and infants to improve effective public health measures.

To the best of our knowledge, there is limited data on the biochemical characteristics and vitamin D status of refugee newborns hospitalized in neonatal intensive care units (NICU) in Turkey. However, VDD is mainly listed in the etiology of late-onset hypocalcemia in the neonatal period, as a probable cause of hypocalcemia in the early period is determined in our study.

Methods

Compliance with Ethical Standards

This study was approved by the University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee with protocol number 2020-173 and date: 09.09.2020. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from the patients' parents.

Study Design and Participants

A total of 2,346 newborns were born in the hospital during this study period, and 673 babies were admitted to the NICU. Among these babies, 155 infants were diagnosed with early-onset hypocalcemia; 15 of them were excluded because of incomplete data for vitamin D levels; and 140 infants were included in the study. These 140 infants were divided into two subgroups and comparatively analyzed (refugee newborns: 94, native newborns: 46) (Figure 1). Our study included newborns admitted to the NICU from June 1 to August 31, 2020, which was the summer period in Turkey. The inclusion criteria were (1) postnatal age at admission ≤3 days; (2) term infants with serum calcium levels <8 mg/dL (2 mmol/L) or ionized calcium levels <4.4 mg/dL (1.1 mmol/L); and (3) preterm infants with serum calcium levels <7 mg/dL (1.75 mmol/L) or ionized calcium levels <4 mg/dL (<1 mmol/L) detected at the maternity ward; and (4) absence of a history of calcium and vitamin D use before hospitalization. The exclusion criteria were as follows: (1) newborns admitted to the NICU for any other diseases such as transient tachypnea of the newborn, meconium aspiration syndrome, acute respiratory distress syndrome, complex congenital heart disease, and congenital malformations; (2) infants born to a preeclamptic mother or mothers using antiepileptic drugs (phenobarbitone, phenytoin sodium); (3) infants having undergone phototherapy, history of receiving diuretics, lipid infusions, or blood transfusions.

Hypocalcemia in asymptomatic babies was detected by routine biochemical tests during follow-up at the maternity ward. Neuromuscular irritability (jitteriness, seizures, exaggerated startle, and myoclonic jerks) and cardiac involvement (prolonged QT interval and/or cardiac rhythm disturbances) were accepted as symptomatic hypocalcemia findings, and infants with these symptoms were admitted to the NICU soon after the symptoms were observed.

Data Collection and Procedures

Anthropometric and clinical findings of the neonates were retrieved from the hospital records, including gestational age, birth weight and height, head circumference, gender, intrauterine growth status, postnatal day at admission, mode of delivery, presence of perinatal asphyxia, calcium normalization day, route of calcium replacement, and length of hospital stay; maternal conditions, including maternal age, number of births, history of using regular vitamin D supplementation, and maternal complications, such as gestational diabetes and preeclampsia. Gestational age of 37 weeks was accepted as preterm birth.

Daily 1200 IU vitamin D maternal intake within the first trimester, independent of vitamin D level, is accepted as regular vitamin D intake during pregnancy, as stated in the Turkish Ministry of Health Support Program (10).

Blood Sampling and Analysis

Venous blood samples were collected at the time of NICU admission, and serum levels of leukocytes [platelet count [$\times 10^3$/L, white blood cell count (10^3 /L)], C-reactive protein (CRP) (mg/L), total calcium, phosphorus, magnesium, ALP, and PTH were measured on the first day. Serum calcium levels were measured again before discharge to evaluate the normalization duration. Serum samples for 25-hydroxy vitamin D [25(OH)D] were maintained at -80 °C and measured using the enzyme immunoassay method (IDS Immunodiagnostic Systems). Neonatal 25(OH)D levels of 12 ng/mL (30 nmol/L) were considered to be VDD, levels between 12 and 20 ng/mL (30-50 nmol/L) were considered to be vitamin D insufficiency, and levels >20 ng/mL (50 nmol/L) were reported as vitamin D sufficiency according to the Global Consensus Recommendations (11).

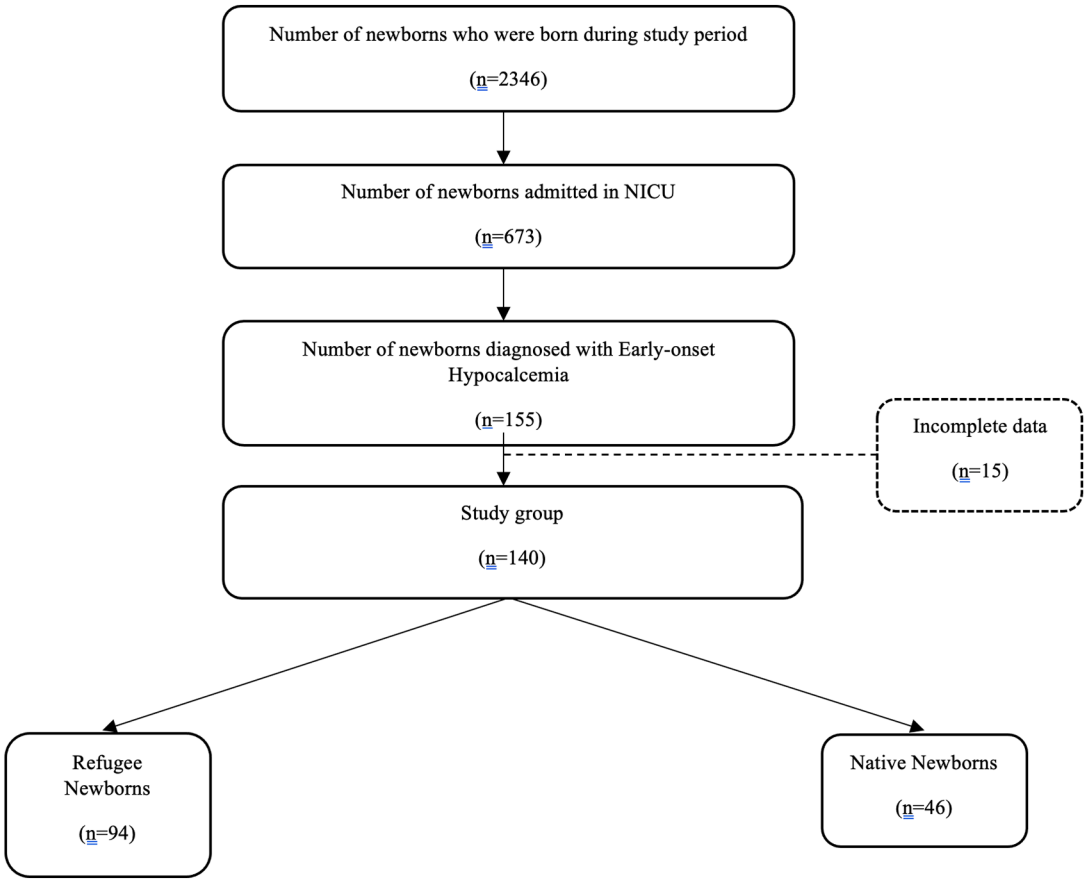

Figure 1. Flow diagram of the study

Statistical Analysis

IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Descriptive statistics are expressed as numbers and percentages for categorical variables. The Kolmogorov-Smirnov test was used to assess the conformity of variables to a normal distribution. If numerical variables were normally distributed, they were expressed as mean ± standard deviation, whereas if numerical variables were non-normally distributed, they were expressed as median (interquartile range: Q1-Q3). To compare categorical variables, the chi-square test was performed, and the comparison of quantitative data between the two groups was assessed using the Mann-Whitney U tests. Statistically significant was accepted as a p-value of less than 0.05.

Results

Study Population and Characteristics

A total of 140 newborns [male: 93 (66.4%), female: 47 (33.6%), with a mean gestational age of 38.1 (±2.0) weeks, a birth weight of 2933 (±665) g, and a birth height of 49.3 (±2.9) cm] were enrolled in the study.

Ninety-four (67.1%) of the babies were Syrian refugee newborns, 46 (32.9%) were native newborns, and 30 of the babies (21.4%) were preterm infants. Gestational age, birth weight, height, head circumference, gender, SGA, appropriate for gestational age, large gestational age ratios, perinatal asphyxia, gravity, parity, gestational diabetes, and maternal vitamin D supplementation use of refugees and native newborns were similar. The rates of prematurity, cesarean section, and maternal age were significantly higher in native newborns (respectively, p=0.022, p=0.001, and, p=0.002). The baseline demographic and laboratory values of the newborns and maternal features are given in Table 1.

Analysis of Vitamin D and Calcium Status

The mean calcium levels of all babies were 7.16 (0.46) mg/dL [minimum-maximum (min.-max.) 5.5-7.9], the median vitamin D levels of babies were 8.36 ng/dL (min.-max. 5.93-11.1), and 79.1% of the infants had VDD and 14.4% had vitamin D insufficiency. No significant difference was found in the prevalence of vitamin D sufficiency, insufficiency, and deficiency between term and

Table 1. Baseline demographics of newborns and maternal features					
Characteristic		**All newborns (n=140)**	**Refugee newborns (n=94)**	**Native newborns (n=46)**	**p-value**
Demographic characteristics					
Gestational age (week), mean (SD)		38.1 (2.0)	38.3 (1.9)	37.7 (2.0)	0.097*
Prematurity, n (%)		30 (21.4%)	15 (16%)	15 (32.6%)	0.024**
Gender, n (%)	Male	93 (66.4%)	66 (70.2%)	27 (58.7%)	0.175**
Birth weight (gr), Mean (SD)		2933 (665)	2921 (687)	2957 (626)	0.757*
Birth height (cm), mean (SD)		49.3 (2.9)	49.3 (3.1)	49.3 (2.5)	0.909*
Head circumference (cm), mean (SD)		34.5 (3.8)	34.1 (1.7)	35.4 (6.1)	0.06*
Weight for GA, n (%)	SGA	30 (21.4%)	22 (23.4%)	8 (17.4%)	0.715**
	AGA	98 (70%)	64 (68.1%)	34 (73.9%)	
	LGA	12 (8.6%)	8 (8.5%)	4 (8.7%)	
Mode of delivery, n (%)	Cesarian section	72 (51.4%)	42 (44.7%)	30 (65.2)	0.022**
Perinatal asphyxia, n (%)		1 (0.7%)	1 (1.1%)	0 (0%)	0.483**
Maternal features					
Maternal age (years), mean (SD)		26.5 (6.6)	24.8 (5.9)	29.9 (6.6)	<0.001*
Gravity, median (Q1-Q3)		2 (2-3)	2 (1-3)	2 (2-4)	0.195***
Parity, median (Q1-Q3)		2 (1-3)	2 (1-3)	2 (1.75-3)	0.789***
Gestational diabetes, n (%)		5 (3.6%)	3 (3.2%)	2 (4.3%)	0.664**
Maternal vitamin D supplementation, n (%)		18 (12.9%)	12 (12.8%)	6 (13%)	0.963**

*t-test, **Chi-square test, ***Mann-Whitney U test
SD: Standard deviation, GA: Gestational age, SGA: Small gestational age, AGA: Appropriate gestational age, LGA: Large gestational age

preterm infants. Symptomatic hypocalcemia developed in 5 patients (3.0%) during hospitalization, and all of these babies were refugees. Calcium, phosphorus, PTH, vitamin D, magnesium, ALP, leukocytes, platelets, and CRP levels were similar in refugee and native newborns. The mean hospital stay was 9.1 (0.6) days. Refugee babies had a longer hospital stay compared to the natives (p=0.022). Calcium was applied more frequently by the intravenous route, and the duration of calcium normalization was longer in refugee newborns than in native newborns (respectively, p=0.048 and p=0.026) (Table 2). Calcium and vitamin D levels of refugee and native newborns are shown as scatter plots (Figure 2).

Discussion

In this study, vitamin D levels, biochemical status, and clinical characteristics of neonates with early-onset hypocalcemia were evaluated, and vitamin D levels and biochemical status were compared between refugee newborns and native newborns. Vitamin D deficiency was found in every eight in ten newborns with early-onset hypocalcemia in our study. The prevalence of VDD among neonates was reported at 61% in a recently published meta-analysis including eighteen studies (12), and in the studies conducted in Iran (13) and Jordan (14), the results for VDD were found to be over 90%. A meta-analysis of vitamin D prevalence in South European countries showed an increased trend in newborns, besides, there were different rates in different countries. In particular, VDD in newborns from Spain was found to be lower compared to other countries, but Turkey also had higher rates (15). A multicenter study with 61 centers from Turkey reported that the prevalence of VDD was 86.5% in newborns with late-onset hypocalcemia (16). A recent meta-analysis of 11 studies including 452 newborns with hypocalcemia and 2,599 newborns with normal serum calcium levels revealed that VDD in newborns may be related to the higher prevalence of hypocalcemia, and maternal VDD may also be a risk factor for neonatal hypocalcemia. This meta-analysis indicates that newborns with VDD have a higher risk of hypocalcemia, and maternal vitamin D levels play a crucial role in this association (17). Our study is valuable because the data of patients with early neonatal hypocalcemia have not been evaluated before, whereas studies frequently report hypocalcemia related to VDD during all neonatal periods.

The vitamin D level of a newborn is closely related to maternal vitamin D level, breastfeeding, and sunlight exposure duration (7,8). The prevalence of VDD was 74.5% and 88.9%, and insufficiency was 17% and 8.9% in the refugee and native neonates hospitalized in the NICU, respectively (p=0.132). In a study by Abdelmageed et al. (18), 365 pregnant women were prospectively

Table 2. Laboratory findings and clinical features of the newborns					
		All newborns (n=140)	**Refugee newborns (n=94)**	**Native newborns (n=46)**	**p-value**
Laboratory finding					
Baseline calcium (mg/dL), mean (SD)		7.16 (0.46)	7.12 (0.47)	7.23 (0.45)	0.188*
Phosphorus (mg/dL), Mean (SD)		5.7 (1.1)	5.8 (1.2)	5.6 (0.9)	0.253*
Magnesium (mg/dL), Median (Q1-Q3)		2.00 (1.70-2.00)	1.95 (1.70-2.00)	2.00 (1.70-2.00)	0.849**
ALP (U/L), median (Q1-Q3)		201 (167-246)	208 (166-249)	186 (169-242)	0.706**
Calcium at discharge (mg/dL), mean (SD)		9.18 (0.68)	9.23 (0.66)	9.08 (0.69)	0.213*
Leucocyte count (10^3/L), mean (SD)		16770 (7200)	16657 (7859)	17007 (5796)	0.767*
Platelet count (10^3/L), mean (SD)		257 (86)	252 (81)	267 (96)	0.392*
C-Reactive Protein (mg/L), Median (Q1-Q3)		1.42 (0.24-11.92)	1.75 (0.32-12.2)	1.20 (0.20-11.60)	0.359**
Parathormone (U/L), median (Q1-Q3)		74.8 (46.8-114.3)	77.6 (49.4-112.7)	66 (42.9-146.8)	0.838**
25 OH Vitamin D (µg/L), Median (Q1-Q3)		8.36 (5.93-11.17)	8.57 (6.29-12.55)	7.99 (5.58-10.58)	0.317**
	Deficiency, n (%)	110 (79.1%)	70 (74.5%)	40 (88.9%)	0.132***
	Insufficiency, n (%)	20 (14.4%)	16 (17%)	4 (8.9%)	
	Sufficiency, n (%)	9 (6.5%)	8 (8.5%)	1 (2.2%)	
Clinical features					
Number of symptomatic hypocalcemias cases, n (%)		5 (3.6)	5 (5.3)	0 (0)	0.172***
Route of calcium replacement, n (%)	IV + PO	17 (12.1)	15 (16)	2 (4.3)	0.048***
Calcium normalization duration (day), median (Q1-Q3)		4.0 (3.0-5.0)	4.0 (3.0-5.0)	3.0 (2.5-4.0)	0.026**
Length of stay at hospital (day), median (Q1-Q3)		9.0 (7.0-11.0)	9.0 (7.0-12.0)	7.5 (6.0-9.2)	0.002**

*t-test, **Mann-Whitney U test, ***Chi-square test, ALP: Alkaline phosphatase, IV: Intravenous, PO: Per oral

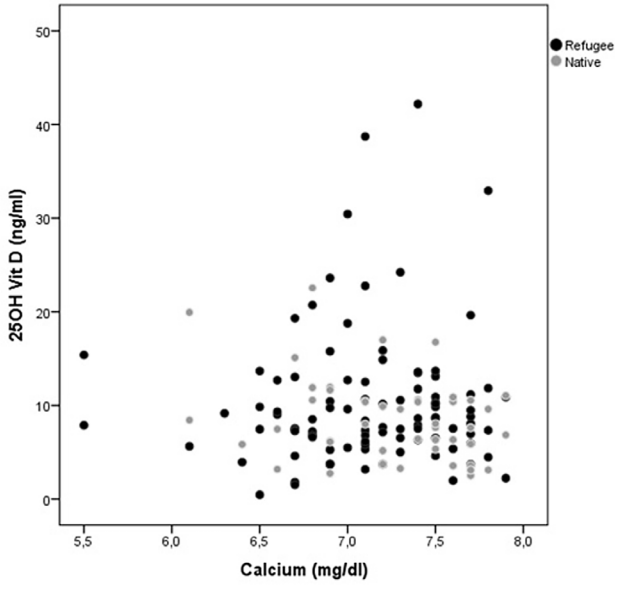

Figure 2. Vitamin D and calcium levels in refugee and native newborns

evaluated in terms of vitamin D levels, and 57.7% of the participants had VDD. They reported that vitamin D supplementation at a daily dose of 1000 IU may not adequately address VDD (18). The study of Kareem Mohammed (19) also revealed a significant prevalence of VDD among pregnant women, and the incidence of VDD was 56.0% among pregnant participants. In our study, the VDD of both refugee and native mothers was similar. Maternal vitamin D supplementation was 12.8% in the refugee group and 13.3% in the native newborns. Besides, no significant difference was found in the vitamin D supplementation of mothers, suggesting similar socioeconomic levels and poor access to public health programs. The rate of covered clothing was also frequent in both groups' mothers, and the parents of both refugee and native newborns were living in the same region, which may explain the similar sun exposure of the mother and baby dyad. Fakhoury et al. (20) examined the relationship between various measures of 25(OH)D status in maternal and neonatal populations and their associations with neonatal outcomes in a sunny Mediterranean region. The study found a link between maternal total and free 25(OH) D levels and all forms of neonatal 25(OH)D levels being positively related. This suggests that maternal vitamin D

levels may have a big effect on the vitamin D status of the newborn (20). Blarduni et al. (21), from Spain, also conducted a study that measured vitamin D levels in 745 mothers and in the umbilical cord blood of 560 newborns. Multiple pregnancies and non-European origin were found to be risk factors for maternal hypovitaminosis, whereas maternal supplementation, physical activity, and sun exposure had a preventive effect.

Hypocalcemia, hyperphosphatemia, and elevated levels of ALP and PTH are the main laboratory findings of VDD. In some cases, only hypocalcemia can be a manifestation of VDD. The mean calcium levels of refugee and native newborns were 7.1 mg/dL and 7.2 mg/dL, respectively. Parathormone, ALP, and phosphorus levels were found within the normal range in both groups, which may be explained by the fact that if VDD was not detected and persisted for a long time, it would increase the levels of these parameters. In a case report including 2 newborns with early-onset hypocalcemia who presented with hypotonia, lethargy, and pathologic tremors, vitamin D levels were low, but PTH was found to be high in one newborn, and both maternal vitamin D levels were deficient. A possible association between ethnicity, maternal vitamin D levels, and neonatal serum calcium levels has been shown; therefore, maternal vitamin D levels and neonatal calcium levels may vary depending on the maternal phototype or cultural factors (22).

Study Limitations

We cannot fully present all maternal biochemical statuses, including vitamin D, calcium, magnesium, phosphorus, and PTH levels, because of financial issues that affect neonatal calcium levels and are thought to be associated with early-onset neonatal hypocalcemia. The sunlight exposure rates of the mothers included in the study were not known, but all pregnancy periods were in similar seasons in both groups. Babies born during the summer period were included in the study. Although a limited number of newborns were enrolled in our study, we showed that VDD in newborns also caused early-onset hypocalcemia.

Conclusion

Neonatal VDD in both native and refugee newborns should also be considered for early-onset hypocalcemia. Maternal regular vitamin D supplementation during pregnancy and lactation and neonatal vitamin D supplementation as soon as after birth should be recommended as the main components of preventive public health policies. Unfortunately, the increased prevalence of VDD is mostly present among women from underdeveloped or developing countries; therefore, health education programs should be established to raise awareness about nutritional and health conditions. Because the majority of hypocalcemic infants have no symptoms related to hypocalcemia, calcium levels must be monitored routinely in newborns born to mothers who have no regular follow-up or vitamin D supplementation. While evaluating the causes of hypocalcemia, treatment for hypocalcemia should be initiated as soon as low calcium levels are obtained. There is a need for further studies to assess the expanded biochemical evaluation of the mother and infant dyad in many patients.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee with protocol number 2020-173 and date: 09.09.2020.

Informed Consent: Written informed consent was obtained from the patients' parents.

Authorship Contributions

Design: B.C., Data Collection or Processing: M.C.U., Analysis or Interpretation: B.C., Literature Search: B.C., M.C.U., Writing: B.C., M.C.U.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

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