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Relationship between vitamin D levels in mother's blood and neonatal umbilical cord with jaundice in neonates

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Abstract

Introduction: In neonates, jaundice is the result of an imbalance between the production and conjugation of bilirubin. Overall, about 60% of full-term neonates experience jaundice in the first week of life. Metabolism of bilirubin and vitamin D occurs in two separate pathways, but because one stage of their production happens in liver, they may affect each other.

Objectives: Diagnostic and therapeutic assays are based only on the opinion of obstetricians and gynecologists. The possible results of this study may help to explain preventive methods. In this study, we will evaluate the relationship between serum levels of vitamin D in the mother's blood and the baby's umbilical cord with the occurrence of neonatal jaundice.

Patients and Methods: This prospective study (cohort) was performed on 110 mothers and their newborns in Firoozabadi hospital in Shahr-e Ray. The sampling method was non-probability convenience. Data including maternal age, nationality, gravidity, gestational age, maternal diseases such as chronic the liver and kidney disease, diabetes mellitus, hypertension and the use of anticonvulsant drugs were obtained from the maternity delivery records and mothers who did not meet the inclusion criteria were excluded at first. Maternal blood samples during labor and neonatal umbilical cord samples were used for assaying the serum levels of 25-hydroxy vitamin D, calcium (Ca) and phosphorus (P), alkaline phosphatase (ALP). All neonates visited for jaundice on days 7 and 14 after birth and tested for bilirubin levels if jaundice was observed

Results: There was no significant difference between neonates with normal and abnormal vitamin D levels in terms of jaundice on day 7 ($P=0.571$). Additionally, there was not a significant relationship between mothers with normal and abnormal vitamin D levels in terms of jaundice on day 7 in their neonates ($P=0.587$). However, a statistically significant relationship between normal vitamin D levels in mothers with jaundice in their neonates on day 14 ($P=0.003$) was detected. There was a significant relationship between normal maternal vitamin D with the incidence of neonatal jaundice on day 14 (relative risk = 0.32). In addition, evaluation of the relationship between normal and abnormal vitamin D levels in neonates with 14th day jaundice revealed no statistically significant relationship between the two groups of normal and abnormal ($P=0.1$). Mean serum ALP concentration in mothers of neonates who did not develop jaundice was significantly higher than mothers of neonates with severe jaundice (bilirubin >15 mg/dL) ($P=0.02$). First minute Apgar score in neonates who had no jaundice or developed mild jaundice was significantly higher than neonates with severe jaundice (bilirubin >15 mg/dL) ($P=0.026$).

Conclusion: Overall, in this study, no significant relationship was observed between neonatal 7th day jaundice with maternal and neonatal vitamin D levels. There was a significant relationship between maternal normal serum vitamin D levels with neonatal 14th day jaundice.

Keywords: Neonatal jaundice, Pregnancy, Vitamin D, Bilirubin

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Introduction

In neonates, jaundice is the result of an imbalance between the production and conjugation of bilirubin (1,2). Overall, about 60% of full-term neonates experience jaundice in the first week of life (3). Although some factors such as incompatibility of blood groups and Rh are considered as important reasons for the occurrence of jaundice, the main reasons for the occurrence of this consequence remain unclear. Liver tissue, as one of vitamin D synthesis centers, has an important role in the conversion of indirect to direct bilirubin and therefore has an important role in the pathophysiology of hyperbilirubinemia (4). Vitamin D levels also have a significant relationship with fetal, maternal and placental health (5). Furthermore, according to a study in 2007, more than 80% of Iranian pregnant women had lower than normal vitamin D serum levels, which would result in a decrease in vitamin D in the fetus (6). Recent studies have shown vitamin D receptors in various tissue cells such as liver and pancreas, brain and prostate, as well as the surface of immune cells such as lymphocytes

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Implication for health policy/practice/research/ medical education

About 60% of full-term neonates develop jaundice in the first week of life. Due to the high prevalence of vitamin D deficiency in mothers, the aim of the present study was to evaluate the relationship between vitamin D levels in maternal blood and neonatal umbilical cord with the incidence of neonatal jaundice. Overall, the high economic costs as well as the psychosocial problems associated with hospitalization of neonates with jaundice have prompted researchers to seek preventive and low-cost treatments for the treatment of neonatal hyperbilirubinemia, noting that about 10% to 50% of the causes of neonatal jaundice in hospitalized newborns remain unknown. At present, there is no uniform program in Iran to evaluate maternal vitamin D levels and to correct probable deficiencies.

and macrophages (7,8). Additionally, in the process of metabolism, vitamin D is converted to 25-hydroxyvitamin D in the liver by the enzyme 25-hydroxylase and then to the active form in the kidneys with the help of 1-alpha hydroxylase (9,10). The metabolisms of bilirubin and vitamin D occur in two separate pathways but may affect each other because one stage of the production of each occurs in the liver. The 25-hydroxylation is one of the basic steps in the production of vitamin D, which is done in the liver. In addition, the liver is the place where bilirubin is conjugated. Overall, the high economic costs as well as the psychosocial problems associated with hospitalization of neonates with jaundice have prompted researchers to seek preventive and low-cost treatments for the treatment of neonatal hyperbilirubinemia, noting that about 10 to 50% of the causes of neonatal jaundice in hospitalized newborns remain unknown. At present, there is no uniform program in Iran to evaluate maternal vitamin D levels and to correct probable deficiencies.

Objectives

Diagnostic and therapeutic assays are based only on the opinion of obstetricians and gynecologists. The possible results of this study may help to explain preventive methods. In this study, we will evaluate the relationship between serum levels of vitamin D in the mother's blood and the baby's umbilical cord with the occurrence of neonatal jaundice.

Patients and Methods

Study design

The present study is a prospective cohort study. The study population included neonates born in Firoozabadi hospital (University Hospital affiliated to Iran University of Medical Sciences in Shahr-e Ray) and their mothers during the three months of winter. The sampling method was non-probability convenience. Initially, 145 mothers and their newborns were selected. Exclusion criteria for neonates were; blood type or Rh incompatibility cause of jaundice, jaundice on the first day of life, Glucose-6-phosphate dehydrogenase(G6PD) deficiency, congenital anomalies, polycythemia, direct hyperbilirubinemia, cephalohematoma, neonatal sepsis and exclusion criteria for mothers were; history of chronic hepatitis, history of kidney disease, history of diabetes mellitus, history of hypertension and history of taking anticonvulsant drugs. Basic data of mothers and neonates obtained from interviews and review of delivery records and the history of probable maternal diseases were identified on this basis. Serum levels of vitamin D, calcium (Ca) and phosphorus (P), alkaline phosphatase (ALP) were assayed by using standard kits (Pars Azmoon). Total and direct bilirubin measurements in icteric neonates were performed in the laboratory of Firoozabadi hospital. At the time of admission of all mothers in the delivery ward, a blood sample is routinely taken and the mother's blood type and Rh are checked. In this study, the same maternal blood sample was used for our evaluations, which included serum levels of 25-hydroxy vitamin D, Ca and P, ALP. After delivery, according to the instructions of the delivery department and operating room, weight, height, head circumference of neonates and type of delivery, problems during labor such as asphyxia and congenital anomalies are determined based on observations and examination by a pediatrician, therefore, features like weight, height, head circumference, and neonatal Apgar scores can be obtained from delivery records. A blood sample is taken from the umbilical cord of all neonates at birth to perform the CBC and blood groups with Rh tests. In our study, this sample, with an approximate volume of 5 cc for the determined tests, which include 25-hydroxy vitamin D, Ca, P and ALP was used and no additional sampling was imposed on the neonates. The samples were sent to Firoozabadi hospital laboratory and frozen and stored at -20°C. Samples were kept in the laboratory for final approval. At the time of discharge from the hospital, all mothers were asked, in order to continue the study, to visit the clinic of Firoozabadi hospital on the 7th and 14th days of childbirth so that their newborns be visited and checked for jaundice. As coordinated, all neonates were visited on the 7th and 14th days of birth, and if jaundice that required the test was observed, a Bilirubin test was requested and a secondary follow-up test was performed on the 14th day. During this period, the hospitalized neonates due to severe jaundice were also followed up and tests related to the time of hospitalization were reviewed and recorded. Follow-up of screening results for outpatients performed and for hospitalized neonates was done by hospital records. Considering that the national screening test for G6PD deficiency is performed for all neonates, in case of G6PD deficiency the neonate was excluded from the study. Neonates with direct hyperbilirubinemia (direct bilirubin >2 mg/dL) were also excluded. All families were followed up by telephone to emphasize and remind the referrals.

According to the sampling method of non-probability convenience, out of 145 neonates and mothers included in the study, 8 neonates due to asphyxia and sepsis, 10 neonates due to incompatibility of maternal and neonatal blood groups, 5 neonates due to hypothyroidism, one neonate due to G6PD deficiency were excluded from the study and also 11 cases due to the mother's unwillingness to continue cooperation. Finally, in this study, 110 mothers and their neonates (220 in total) were selected based on pre-determined criteria and the assays performed on their samples. Conscious consent was obtained from all mothers before entering the project. Based on the results of the clinical examination and Bilirubin test, neonates who did not need to be tested (jaundice in the face or upper chest) or did not have jaundice at all or had bilirubin <10 mg/dL, were classified in the group "no jaundice"; bilirubin=10-15 mg/dL in the group of "mild jaundice"; and neonates with bilirubin> 15mg/dL in the group of "severe jaundice".

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Iran University of Medical Sciences approved this study. The institutional ethical committee at Iran University of Medical Sciences approved all study protocols (ethics IR.IUMS.FMD.REC.1397.036). Accordingly, written informed consent was taken from all participants before any intervention. This study was extracted from the doctoral dissertation in family medicine specialty by Forough Mokhtari at this university (Thesis #757).

Data analysis

Central tendency for quantitative and qualitative parameters was expressed as mean and standard deviation (mean ±SD) and number (percent), respectively. Independent t-test was used to compare quantitative parameters between two groups and chi-square test was used to compare qualitative parameters. In case of comparison between more than two groups we used ANOVA and Post Hoc (Scheffe) test. Additionally, P-value less than 0.05 was considered significant and SPSS version 20 was used for statistical analysis.

Results

In this study, 110 mothers and their neonates (220 in total) remained until the end of the study. Table 1 depicts basic data of mothers and neonates.

According to the findings of Table 2, out of 110 mothers studied, 67 mothers (60.9%) had serum vitamin D levels less than 20 (deficient) and 16 mothers (14.5%) had vitamin D levels in the range of 20-29.9 (insufficient). In 27 mothers (24.5%), serum levels of vitamin D were 30 and higher (sufficient). In total, 27 mothers (24.5%) had normal vitamin D levels and 83 mothers (75.5%) had abnormal (deficient or insufficient) vitamin D levels. Additionally, out of 110 neonates studied, 75 neonates

Table 1. Demographic and laboratory data of mothers and neonates

Parameter	Mean (SD)	Min-Max
Mother's age (y)	26.48 (5.6)	18-44
Birth weight (g)	3384.14 (396.83)	2530-4300
Height at birth (cm)	49.78 (2.29)	41-56
Head circumference at birth (cm)	34.98 (1.50)	29.5-39
Gestational age (wk)	39.04 (1.01)	37-41
Apgar first minute	8.95 (0.26)	
Apgar fifth minute	9.99 (0.09)	
Maternal serum vitamin D (ng/mL)	19.453 (16.7252)	3.0-100.0
Maternal serum Ca (mg/dL)	9.132 (0.3563)	8.1-10.0
Maternal serum P (mg/dL)	3.738 (0.8088)	2.2-9.9
Maternal ALP (U/L)	454.596 (197.2167)	4.0-1562.0
Neonatal umbilical cord vitamin D (ng/mL)	16.519 (13.9769)	3.0-87.1
Neonatal umbilical cord Ca (mg/dL)	9.915 (0.4911)	8.6-11.2
Neonatal umbilical cord P (mg/dL)	4.986 (0.7343)	3.3-7.2
Neonatal umbilical cord ALP (U/L)	462.690 (182.4249)	15.6-998.0
	No. (%)	
Education of mothers		
Illiterate	42 (38.2)	
Not completed High school	41 (37.3)	
High school Diploma	24 (21.8)	
Associate degree	1 (0.9)	
Bachelor degree	1 (0.9)	
Master degree	1 (0.9)	
Gender		
Male	57 (51.8)	
Female	53 (48.2)	
Gravidity maternal		
1st	35 (31.8)	
2nd	32 (29.1)	
3th	18 (16.4)	
4th	14 (12.7)	
5th	8 (7.3)	
6th	2 (1.8)	
7th	1 (0.9)	
Nationality of mothers		
Iranian	56 (50.9)	
Afghani	54 (49.1)	
Neonatal feeding	98 (89.1)	
Breastfeeding	1 (0.9)	
Formula	11 (10)	
Combined	98 (89.1)	
Delivery method		
NVD	93 (84.5)	
C/S	17 (15.5)	

CA, calcium; P, phosphorus; ALP, alkaline phosphatase; NVD, Normal vaginal delivery; C/S, Cesarean section.

Table 2. Classifying of neonates based on jaundice and classifying of mothers and neonates based on vitamin D (ng/mL) levels

	No jaundice, No. (%)	Mild jaundice, No. (%)	Severe Jaundice, No. (%)
Classifying of neonates based on 7th day jaundice	66 (60%)	33 (30%)	11 (10%)
Classifying of neonates based on 14th day jaundice	90(82%)	20 (18%)	0 (0%)
Vitamin D levels	No. (%) of mothers	No. (%) of neonates	
0-19.9 (deficient)	67 (60.9%)	75 (68.2%)	
20-20.9 (insufficient)	16 (14.6%)	20 (18.2%)	
30 and more (sufficient)	27 (24.5%)	15 (13.6%)	

(68.2%) had vitamin D levels less than 20 ng/mL (deficient) and 20 neonates (18.2%) had vitamin D levels in the range of 20-29.9 ng/mL (insufficient). In 15 neonates (13.6%), serum levels of vitamin D were ≥30 ng/mL (sufficient). In total, 15 neonates (13.6%) had normal vitamin D serum levels and 95 neonates (86.4%) had abnormal vitamin D serum levels (deficient or insufficient). In terms of other laboratory parameters, 13 neonates (11.8%) had above normal calcium levels and 97 neonates (88.2%) had normal umbilical cord calcium levels. In terms of phosphorus levels, 8 neonates (7.3%) had lower than normal phosphorus levels and 102 (92.7%) had normal phosphorus levels. Additionally, 3 neonates (2.7%) had lower than normal ALP levels and 107 (97.3%) had normal levels. Among mothers, 4 mothers (3.7%) had under normal calcium serum levels and 96 mothers (87.3%) had normal calcium serum level. In terms of phosphorus levels, 7mothers (6.3%) had above normal phosphorus levels, one mother (0.9%) had under normal, and 102 mothers (92.8%) had normal phosphorus serum levels. Moreover, 81 mothers (73.7%) had higher than normal ALP serum levels, 2 mothers (1.8%) had lower than normal levels and 27 mothers (24.5%) had normal levels. Noting that the level of vitamin D ≥30 ng/mL is considered normal, in the study of the relationship between the level of vitamin D in the mother and the neonate in two normal and abnormal states with the occurrence of neonatal jaundice on days 7 and 14, the results are as follows;

The incidence of neonatal jaundice on day 7 in neonates of mothers with vitamin D deficiency was 38.6% and for neonates of mothers with normal vitamin D levels was 44.4%. The incidence of neonatal jaundice on day 7 was 38.9% in neonates with vitamin D deficiency and 46.7% in neonates with normal vitamin D. The incidence of neonatal jaundice on day 14 was 12% in neonates of mothers with vitamin D deficiency and 37% in neonates of mothers with normal vitamin D. The incidence of neonatal jaundice on day 14 was 15.8% in deficient vitamin D neonates and 33.3% in neonates with normal vitamin D.

According to the findings of Table 3, no significant relationship was observed between normal and abnormal vitamin D levels in mothers with the incidence of neonatal jaundice on the 7th day in their neonates ($P=0.587$); In addition, no statistically significant relationship between normal and abnormal vitamin D levels in neonates with the occurrence of neonatal jaundice on the 7th day was seen ($P=0.571$).

However, a significant relationship between normal vitamin D levels in mothers with the incidence of neonatal jaundice on the 14th day in their neonates was seen ($P=0.003$) while relative risk in this case was 0.32. It means, there is a significant relationship between normal maternal vitamin D levels with the incidence of neonatal jaundice in neonates on 14th day. Noting that relative risk is less than1 (ratio of the probability of neonatal jaundice in the exposed group to the probability of neonatal jaundice in the unexposed group), According to the results of the study, vitamin D deficiency in mothers is a protective factor for the occurrence of neonatal jaundice on the 14th day. Besides, no statistically significant relationship between normal and abnormal vitamin D levels in neonates with the incidence of neonatal jaundice on the 14th day was detected ($P=0.1$).

The mean ALP serum levels in mothers of neonates with neonatal jaundice on day 14 was significantly lower than mothers whose neonates did not develop neonatal jaundice ($P=0.02$).

In the study of the relationship between nationality and neonatal jaundice group, a significant relationship was

Table 3. Relationship between vitamin D levels in mothers and newborns with neonatal jaundice occurrence on the 7th and 14th days

		7th day Neonatal jaundice		*P* value	14th day Neonatal jaundice		*P* value
		Positive, No. (%)	Negative, No. (%)		Positive, No. (%)	Negative, No. (%)	
Maternal group vitamin D	Abnormal	32 (38.5)	51 (61.5)	0.587	10 (12)	73 (88)	0.003
	Normal	12 (44.4)	15 (55.6)		10 (37)	17 (63)	
Neonatal group vitamin D	Abnormal	37 (38.9)	58 (61.5)	0.571	15 (15.8)	80 (84.2)	0.10
	Normal	7 (46.7)	8 (53.3)		5 (33.3)	10 (66.7)	

found, so that bilirubin >15 mg/dL was significantly more observed in Iranian individuals ($P=0.02$).

The first minute Apgar score in neonates who did not develop neonatal jaundice or had mild neonatal jaundice was significantly higher than neonates with severe neonatal jaundice (bilirubin >15 mg/dL; $P=0.026$).

Discussion

In this study, which was conducted to evaluate the relationship between vitamin D levels in mothers and neonates with the incidence of neonatal jaundice, no significant relationship was observed between normal and abnormal vitamin D levels in mothers and neonates with the incidence of neonatal jaundice on the 7th day ($P=0.571$, $P=0.587$). However, a significant relationship between normal vitamin D levels in mothers with the incidence of neonatal jaundice on the 14th day was detected ($P=0.003$). There was a relationship between normal vitamin D levels in mothers with the incidence of neonatal jaundice on the 14th day and maternal vitamin D deficiency was a protecting factor against the occurrence of neonatal jaundice on the 14th day (relative risk = 0.32). Moreover, no statistically significant relationship between normal and abnormal vitamin D levels in neonates with neonatal jaundice on the 14th day was found ($P=0.1$).

Considering that the incidence of neonatal jaundice in our study (40%) on the 7th day was lower than expected for term neonates (60%) and on the other hand we had a high prevalence of vitamin D deficiency (86.4%) in neonates, it seems that a larger sample size was required for a more precise study. On the other hand, the neonatal levels of vitamin D were assayed only at birth, and a series of tests in the first and second weeks of birth may provide more accurate data. In order not to impose additional blood sampling on neonates, bilirubin test was not performed for neonates with jaundice of the face and upper chest, and was done only for neonates who had jaundice more than that, hence conducting a similar study with higher sample size and bilirubin testing for all neonates, and repeated vitamin D testing for them can achieve more accurate results. In the study by Maghbooli et al, the mean serum level of vitamin D was 27.8 ± 21.71 (nmol/L) and the prevalence of vitamin D deficiency in mothers was 66.8%, which was lower than our study (75.5%) (6). The study by Zia et al in Shiraz, with the aim of evaluating the effect of maternal vitamin D deficiency on increasing the risk of hyperbilirubinemia in term neonates, with a sample size of 300, showed the relative frequency of low vitamin D levels in pregnant women was 92.3% (11). Furthermore, the relative frequency of severe, moderate and mild vitamin D deficiency in neonates was 33.8%, 51.8% and 7.7%, respectively. Another study reported that 86% of mothers and 75% of their neonates were deficient in vitamin D in winter and 46% of mothers and 35% of their neonates were deficient in vitamin D in summer (12). In the study of Ding et al, the level of the vitamin changes with seasons and its highest serum level is in autumn (13). Therefore, it can be concluded that the season of taking laboratory samples of mothers and neonates can also have effects on the results of the study. In the study of González de Dios et al (14) which was conducted on 61 neonates with pathological hyperbilirubinemia, the rate of increase in bilirubin was significantly higher in summer than in other seasons; however in the cohort study of Sajjadian et al, which performed in Iran for one year with a sample size of 629 neonates, the rate of increase in neonatal bilirubin in the first 24-48 hours of birth was significantly higher ($P<0.001$) in winter than other seasons (15).

Considering the different results in the two cited studies and noting that in our study the low prevalence of neonatal jaundice can effect on results of the study and that the study was done in one season, conducting of similar studies in a longer period, including all seasons can yield more accurate results. In the study of Mehrpisheh et al, the mean serum level of vitamin D in mothers of neonates with jaundice was 14.72 ± 9.60 ng/mL and in the control group was 17.71 ± 12.66 ng/mL, since there was no statistically significant difference between the two groups (16). Furthermore, no significant relationship was seen between vitamin D levels with neonatal jaundice in this study ($P=0.119$) which was against the results of our study about the 14th day neonatal jaundice but was in the line about the 7th day neonatal jaundice, also, in terms of significant relationship, it was not consistent with the study by Mutlu et al. In the study of Mutlu et al (17), which was conducted in 2010-2011 to determine the relationship between vitamin D levels with neonatal jaundice in, the mean vitamin D level in neonates with jaundice was 10.7 ± 4.9 ng/mL and in the control group was 15.7 ± 4.9 ng/mL, while a significant difference between the case and the control groups was found ($P=0.01$). The relationship between these two parameters (bilirubin and vitamin D) was different in the study by Mutlu et al and our study, thereby in this study, unlike our study for 14th day neonatal jaundice, vitamin D levels in neonates with jaundice were less than neonates without jaundice. Accordingly in the study by Aletayeb et al, comparing the serum levels of vitamin D between newborns with neonatal jaundice and the control group and their mothers showed that the serum vitamin D levels in both groups were not related to their serum bilirubin levels (18). Although the mean serum Vitamin D levels were significantly lower in neonates with jaundice compared to the control group, the result of this study in terms of lack of significant relationship between mean vitamin D serum levels in newborns with and without 7th day neonatal jaundice was compatible with the result of our study. In the study of Mehrpisheh et al, the mean serum level of vitamin D in icteric neonates was 10.76 ± 8.60 ng/mL and in the control group was 14.88 ± 11.38 ng/mL (16). Although the mean serum level of vitamin D in neonates with jaundice was lower than the control group, no statistically significant

difference was seen, and no significant relationship was observed between vitamin D levels and neonatal jaundice in their study ($P=0.119$). Their result was also against the result of our study on 14th day neonatal jaundice but was compatible with the result of 7th day neonatal jaundice and was not consistent with the results of the study by Mutlu et al in terms of significant relationship. In the study of Siu et al, the type of neonatal feeding had a significant effect on the mean serum level of total bilirubin in neonates. Thereby in newborns who used breast milk, the mean total bilirubin was significantly higher than the two groups fed by formula or combination of formula and breast milk (19). In our study, the type of neonatal feeding did not affect the incidence of neonatal jaundice, but considering the population of the study and noting the fact that just one newborn was exclusively fed by formula, more populated studies focused on the type of feeding are needed to evaluate the significant results on relation of feeding with development of hyperbilirubinemia and jaundice in neonates. Other risk factors for neonatal jaundice such as first day jaundice, hemolysis due to blood incompatibility, G6PD deficiency, infection, and cephalohematoma were considered as exclusion criteria at the beginning of our study.

Conclusion

Overall, limited studies have been conducted in this regard, which often are of the case-control types. Noting that in the present study, the level of vitamin D was assayed only at birth, it is suggested that in the future studies, at the same time with the assays of bilirubin, the level of vitamin D be checked again with more monitoring and correlations. Considering our results and comparing them with the consistent and different studies, it seems that conducting more prospective studies with a higher sample size can be helpful.

Limitations of the study

Follow-up of neonates on days 7 and 14 and coordination with mothers to visit neonates was a time-consuming and difficult process. Laboratory coordination was difficult for accepting neonatal samples within 24 hours of delivery and for simultaneous preparation of maternal and neonatal specimens by delivery room personnel. Accurate evaluation of neonatal exclusion criteria required repeated follow-up and comprehensive examination of neonates, which would be removed if the family did not cooperate. PTH measurement in the initial planning to start the study was part of the tests requested for mother and neonate, but due to the lack of relevant laboratory kit in Firoozabadi hospital, it was coordinated with another hospital laboratory. The relevant hospital didn't accept samples without national ID (including sample Afghani mothers and neonates) and eventually PTH measurements were eliminated due to the high cost in a private laboratory.

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Authors' contribution

ZS, MN, MBi, MBa, FM were the principal investigators of the study. They participated in the concept and design, revision of manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript and approved their cooperation.

Conflicts of interests

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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