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Original Article

Assessment of vitamin B12 and homocysteine levels in pregnant women admitted for delivery and cord blood samples of their newborn babies: a multicenter study

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ABSTRACT

Background. Vitamin B12, an indispensable micronutrient, is pivotal in numerous physiological processes, with particular significance during pregnancy and fetal development. The increasing adoption of vegetarian diets and the economic challenges associated with accessing animal-based food sources contribute to the prevalence of vitamin B12 deficiency. This study aims to examine the levels of vitamin B12 and homocysteine in pregnant women upon admission for delivery and to analyze corresponding cord blood samples from their newborn infants in a substantial sample within the Istanbul metropolitan area.

Materials and Methods. This cross-sectional multicenter study included women aged ≥16 years admitted for delivery and their newborns ≥34 weeks. The demographic data and the results of complete blood counts within the previous 24 hours before birth were recorded. Vitamin B12 and homocysteine levels were measured in maternal and cord blood samples. The study parameters were compared between the groups based on the mothers' and babies' homocysteine and vitamin B12 levels.

Results. The study included 832 pregnant women and 832 neonates. Anemia affected 36% of pregnant women, with a higher frequency in mothers with vitamin B12 deficiency. Seventy-eight mothers and 48.9% of neonates showed Vitamin B12 levels below 200 pg/mL, while elevated homocysteine levels were observed in 30% of mothers and 26% of neonates. Maternal vitamin B12 deficiency was significantly correlated with cord blood B12 deficiency and elevated homocysteine. The median cord blood vitamin B12 level was inversely correlated with the number of previous pregnancies.

Conclusion. Vitamin B12 deficiency is extremely common in pregnant women before delivery, significantly correlating to cord blood homocysteine and vitamin B12 levels. However, homocysteine alone is not a reliable marker for maternal vitamin B12 status. Implementing strategies to detect vitamin B12 deficiency and supplying

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adequate vitamin B12 supplementation during pregnancy holds the potential to enhance maternal and neonatal health in Türkiye.

Key words: vitamin B12, homocysteine, pregnancy, cord blood.

Vitamin B12 (VB12) plays an essential role in various physiological processes, particularly during pregnancy and fetal development.[1] However, VB12 deficiency has become an important public health problem all over the world.[2] The limited consumption of animal foods owing to socioeconomic barriers, and ethical or health considerations contribute to the global increase in the prevalence of inadequate VB12 status.[3,4] During infancy, neonatal VB12 status reflects that of the mothers, in other words, babies born to mothers with VB12 deficiency are also born with insufficient VB12 stores[5], and clinical findings of VB12 deficiency, such as hypotonia, convulsions, growth retardation, anemia, and brain atrophy might become manifest during infancy due to insufficient levels of VB12 in breast milk.[6,7]

VB12 deficiency is generally defined as a VB12 level of less than 200 pg/mL, with threshold levels varying amongst laboratories and studies.[8] However, the signs and symptoms of early VB12 deficiency might be subtle, necessitating more sensitive diagnostic tests. Serum methylmalonic acid (MMA) and homocysteine are recognized as functional biomarkers for assessing VB12 deficiency[9], even in the absence of megaloblastic anemia.[8] A positive correlation between the elevated levels of homocysteine and the development of neurological dysfunction in infants due to VB12 deficiency has been reported as well.[6] However, the lack of agreement on cutoff values for each of the biomarkers continues to be a concern in diagnosing VB12 insufficiency.[10,11]

The objective of this study was to assess VB12 and homocysteine levels in a large group of pregnant women and to evaluate the impact of maternal VB12 status on the VB12 and homocysteine levels in newborns in Türkiye.

Materials and Methods

Healthy pregnant women aged ≥ 16 years admitted for delivery and their healthy babies ≥34 weeks were included in the study. Pregnant women under 16 years of age, babies requiring intensive care admission, babies born with anomalies, and twin pregnancies were not included.

This cross-sectional study was conducted in three tertiary centers in Istanbul. The demographic characteristics, namely, maternal age, mode of delivery, number of previous pregnancies, abortions and births, and drugs used during pregnancy, the height and weight of the neonates were recorded. The gestational week was determined by the last menstrual date or fetal ultrasonography. Whole blood count analysis was performed within 24 hours before delivery. Blood samples for VB12 and homocysteine from mothers (within one hour before delivery) and cord blood of babies were collected from September 2020 to September 2021. The samples were centrifuged, and the sera were stored at -20°C until the day of analysis.

VB12 analysis was performed by the "ECLIA" (electrochemiluminescence immunological test) method in COBAS-E immunological test analyzer. Homocysteine analysis was performed by the chemiluminescence method in Immulite 2000 device.

VB12 levels lower than 200 pg/mL were considered as a deficiency.[12] Homocysteine greater than 10 μmol/L was considered elevated and accepted as a reflection of VB12 functional status.[13,14] Study parameters were compared between groups dichotomized according to maternal VB12 (cut-off value of 200 pg/mL) levels.

Anemia is defined as a hemoglobin concentration of less than 110 g/L according to the World Health Organization Criteria for pregnant women.[15]

All study participants provided written informed consent. The research complied with all the relevant national regulations, and institutional policies, in accordance with the tenets of the Helsinki Declaration, and was approved by the author's institutional review board or equivalent committee (ethical approval number and date: 3132-2/2/2021).

Data were analyzed by SPSS statistical software v.22 program. Descriptive statistics were expressed as mean and standard deviation (SD), median and range for continuous variables, and number and percentage for categorical variables. The chi-square test was used to assess for comparisons of categorical variables between groups. One-way ANOVA and Mann-Whitney U tests were used for the comparison of groups with symmetrical and asymmetrical distribution, respectively. A p-value <0.05 was considered statistically significant.

Results

Two hundred fifty-eight patients were excluded from the study because they did not meet the research inclusion criteria, had insufficient information, or had hemolytic serum samples. Vitamin B12 and homocysteine levels in 832 pregnant women and cord blood samples that met the study criteria were assessed.

The study included 615 Turkish and 217 foreign pregnant women, mostly refugees from Syria. The median age of the study population was 28.1 (range 16.4-51.2) years. Eight percent of the pregnant women were under the age of 20, 25% were between the ages of 21 and 25, 29% were between the ages of 26 and 30, and 38% were between the ages of 31 and over. The average number of pregnancies per woman was three (1-9). 17, 53, 25, and five percent of women had first, 2-3, 4-5, and 6 or more pregnancies, respectively. The mean gestational age was 38.7±1.4 weeks. 46% of the pregnant women had vaginal delivery whereas 54% had Cesarean (C)-sections (Table I).

Sixty-seven percent of mothers received antenatal supplemental folic acid, while 75% received other supplements containing iron and/or multivitamins. 10.6% of pregnant women smoked during pregnancy. Thirty-six percent of the pregnant women were anemic, i.e., had hemoglobin less than 11 gr/dL before delivery. The frequency of anemia was higher in women with VB12 deficiency (p=0.00007). Twenty-one percent, 78%, and 1% of the women had mean corpuscular volume (MCV) values <80 fl, between 80 and 100 fl, and ≥100 fl, respectively.

The mean VB12 level of the pregnant women was found to be 157±75.3 pg/mL (range 27.3-882). Maternal VB12 levels revealed that 78.2% of pregnant women were VB12 deficient (<200 pg/mL), with 51.9% having VB12 levels less than 150 pg/mL. The mean homocysteine level of

Table I. Demographic characteristics of pregnant women.

Parameters	Results
Maternal age, yr, median (min-max)	28.1 (16.4-51.2)
Number of pregnancies, median (min-max)	3 (1-9)
Number of deliveries, median (min-max)	1 (0-8)
Time elapsed since the last birth, yr, mean±SD	3.69± 2.35
Duration since discontinuation of last breastfeeding episode, yr, mean±SD	2.69 ±2.32
Duration of gestation, days, mean±SD	270.81±11.32
Mode of delivery (Number of vaginal birth / Cesarean section)	388/444

SD: standard deviation.

the pregnant women was 8.56±5.51 (range 1.9-51.0) μmol/L while homocysteine levels were elevated in 26% of pregnant women, showing a functional deficiency (Table II).

According to the cut-off VB12 level of 200 pg/mL, maternal age, nationality, number of previous pregnancies and deliveries, time passed since the last birth, breastfeeding, gestational age, and history of smoking and receiving folate supplements during pregnancy were not statistically different between groups with and without VB12 deficiency (p>0.05). Vaginal deliveries and iron/multivitamin supplementation were associated with higher VB12 levels in the mothers (p=0.04 and p=0.01, respectively). Pregnant women who received antenatal iron/multivitamin supplementation had a mean VB12 level of 161.5 ± 75.3 pg/mL, compared to 145.1 ± 75.0 pg/mL in those who did not. Mothers with VB12 levels above 200 pg/mL had higher hemoglobin and hematocrit, higher MCV (within normal range) as well as lower homocysteine values, while their newborns had higher VB12 and lower homocysteine levels in their cord blood (p<0.05) (Table III).

The mean weight of the babies was 3271±435 g whereas the mean length was 49.5±2.2 cm. Mean cord blood VB12 and homocysteine levels were 234.7±13.2 pg/mL (range 30.6-971) and 9.13±5.75 (range 2-51) μmol/L, respectively. 48.9% of newborns were VB12-deficient (200 pg/mL), while homocysteine levels were elevated in 30%.

Vitamin B12 deficiency and elevated homocysteine levels were more frequent in the cord blood of babies born to mothers with VB12 deficiency (p=0.001, Table III). However, 44% of babies born to mothers with a VB12 level of <200 pg/mL had normal VB12 levels (Fig. 1). Cord VB12 levels negatively correlated with the increasing number of previous pregnancies (p=0.036) (Fig. 2). A significant relationship was found between both cord blood VB12 deficiency and maternal VB12 and homocysteine levels. The birth weight significantly correlated with cord blood VB12 levels, but the difference in mean birth weight between babies with and without VB12 deficiency was only 67 grams. The relationship between the gender of the baby and VB12 level was nonsignificant.

Discussion

Our study represents the most comprehensive evaluation of VB12 status in mothers and their newborn infants conducted in our country. The main outcome is that B12 deficiency is extremely common in pregnant women before delivery and that cord blood VB12 and homocysteine levels correlate strongly with maternal VB12 stores.

Table II. Vitamin B12 and homocysteine levels of babies and pregnant women.

Parameter	Maternal	Cord blood
Vitamin B12 , pg/mL (mean±SD)	157±75.3	234.7±13.2
Distribution of cases	78.2% deficient 21.8% sufficient	48.9% deficient 51.1% sufficient
<150	432 (51.9%)	230 (27.6%)
150-200	219 (26.3%)	177 (21.3%)
201-300	151 (18.1%)	259 (31.1%)
>301	30 (3.7%)	166 (20%)
Homocysteine , μmol/L (mean±SD)	8.56±5.51	9.13±5.75
≤10 (normal)	622 (74%)	589 (70%)
>10 (elevated)	210 (26%)	243 (30%)
VB12 <200 pg/mL and Homocysteine >10 μmol/L	179 (21%)	183 (21%)

SD: standard deviation.

Table III. Comparison of study parameters according to vitamin B12 status of mothers.

Study parameter	Maternal VB12 <200 pg/mL (n=652)	Maternal VB12 ≥200 pg/mL (n=180)	p-value
VB12 level, pg/mL, mean ± SD	129.1±40.4	261.6±81.2	0.000
Maternal age, yr, median (range)	27.8 (16.9-51.2)	29.25 (16.4-45.5)	0.06
Turkish / foreign nationality, n (%)	477 (73.1%) / 175 (26.9%)	138 (76.7%) / 42 (23.3%)	0.093
Number of pregnancies, median (range)	3 (1-9)	2 (1-9)	0.27
Number of births, median (range)	1 (0-8)	1 (0-8)	0.23
Number of abortus, median (range)	0 (0-5)	0 (0-5)	0.73
Time since last birth, yr, median (range)	3 (1-17)	3 (1-14)	0.73
Time since last breastfeeding, yr, median (range)	2 (0-15)	2 (1-12)	0.38
Folate usage (%)	66%	71%	0.28
Iron/other vitamin supplements (%)	73%	82%	0.01
Smoking (%)	10.1%	13%	0.17
Gestational age, wk, mean ± SD	38.7±1.3	38.6±1.4	0.33
Vaginal delivery / C/S, n (%)	292 (44.8%) / 360 (55.2%)	96 (53.3%) / 84 (46.7%)	0.04
Male / female infant, n (%)	350 (53.7%) / 302 (46.3%)	84 (46.7%) / 96 (53.3%)	0.10
Birth weight, g, median (range)	3282 (1735-4900)	3225 (2060-4445)	0.09
Birth height, cm, median (range)	50 (34-56)	50 (43-54)	0.24
Hemoglobin, g/dL, median (range)	11.3 (5.7-15,5)	11.8 (7.9-14.5)	0.00007
Hematocrit, %, median (range)	34 (21-46)	35 (25-43)	0.001
MCV, fL, median (range)	85 (59-106)	88 (62-106)	0.002
Leucocyte, /mm^3, median (range)	10600 (4430-31940)	10840 (5930-22080)	0.21
Neutrophil, /mm^3, median (range)	7890 (2000-29690)	7940 (3850-19560)	0.18
Lymphocyte, /mm^3, median (range)	1950 (210-8500)	1850 (760-4160)	0.13
Platelet, /mm^3, median (range)	222000 (45000-591000)	215000 (87000-547000)	0.20
Maternal homocysteine, µmol/L, median (range)	7.6 (1.9-51.0)	6.82 (2.0-39.3)	0.08
Cord VB12, pg/mL, median (range)	187 (40-971)	273 (30.6-906.7)	0.0001
Cord homocysteine, µmol/L, median (range)	8.02 (2.0-51.0)	6.77(2.0-47.9)	0.0001

C/S: Caesarean section, IQR: interquartile range, MCV: mean corpuscular volume, SD: standard deviation, VB12: vitamin B12

In our study, eight percent of pregnant women were under the age of 20, and half were second or third pregnancies. Our study's average age of pregnant women was comparable to other studies conducted in our country.[11,16,17] Although the age of pregnant women deficient in VB12 was younger than that of pregnant women with adequate levels, the difference was not statistically significant. Likewise, the number of pregnancies did not affect VB12 status. Beyoglu et al. related VB12 insufficiency to younger age at pregnancy, particularly between 18 and 24 years, and to gravida three or more.[18] In a study conducted in Türkiye's Southeastern Anatolia region, where the marital age is generally lower, 16% of pregnant women were under the age of 20, while the frequency of first pregnancies was higher than that of our study.[16] The age of the pregnant women and the number of pregnancies did not influence the VB12 status as well. The fact that Istanbul has the highest marital age in Türkiye and the southeastern region of Türkiye has the highest birth rate might elucidate the variations observed in our findings compared to other studies.[19]

Twenty-one percent of the pregnant women had microcytosis in their whole blood count. Microcytosis rates were similar in other studies conducted in our country.[20] Also, around two-thirds of pregnant women had anemia, with a

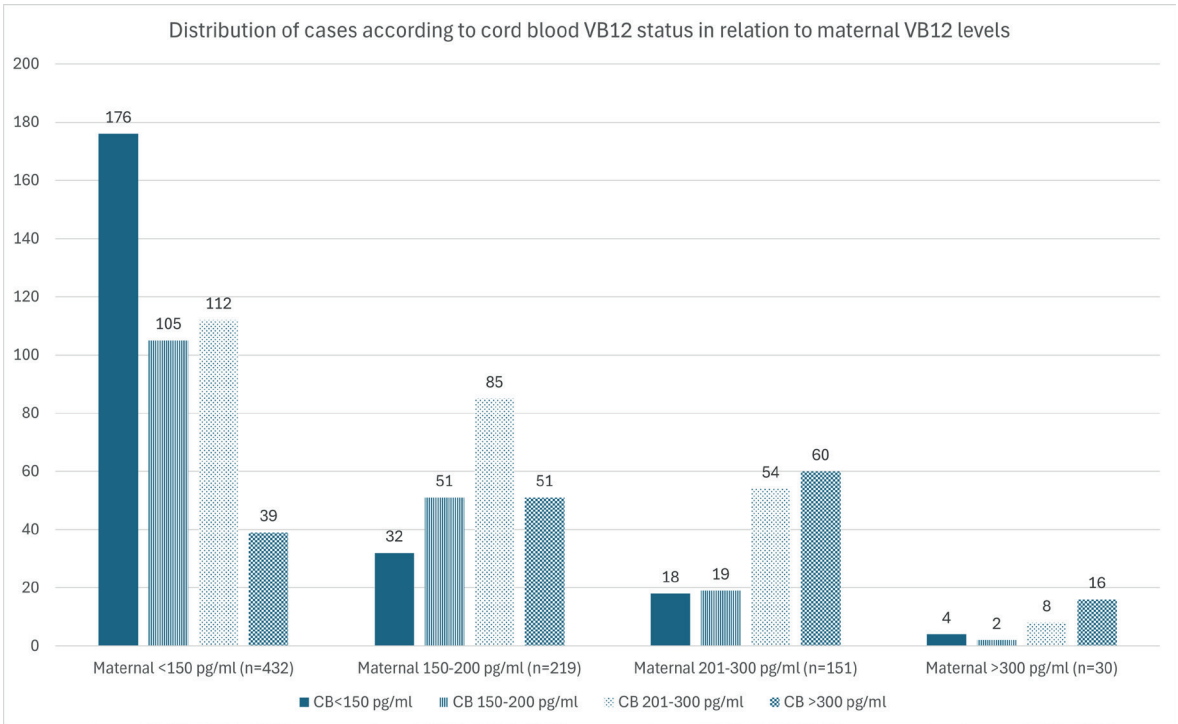

Fig. 1. Cord blood vitamin B12 and maternal vitamin B12 status relationship.

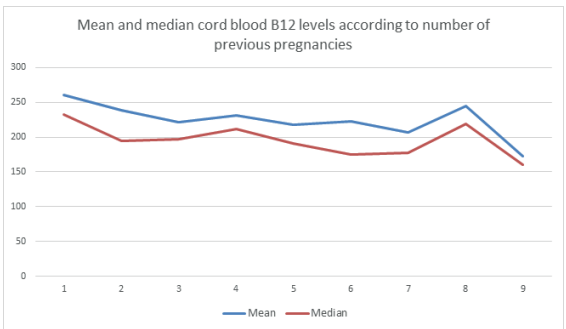

Fig. 2. Cord blood vitamin B12 levels according to number of previous pregnancies (pg/mL).

higher frequency in VB12-deficient mothers. Only 12 out of 832 patients had MCV ≥100 fl and four of 12 had anemia. In addition, MCV values were lower in women with VB12 deficiency, contrary to what would be expected, i.e., macrocytosis. These findings might be attributed to a possible concomitant iron deficiency in our study population. The ferritin levels were not measured in our study; therefore, an objective interpretation of this issue is impossible. These findings also highlight the fact that normal hemogram results in pregnant women were not a reliable indicator of VB12 insufficiency, as Roumeliotis et al. reported.[21]

The mean VB12 levels of the pregnant women in our study were quite low and VB12 deficiency afflicted approximately three-quarters of the pregnant population, with half suffering from levels below 150 pg/mL. Among the studies conducted on VB12 deficiency, there is no consensus on the threshold VB12 value that should be used to define the deficiency. Some studies consider VB12 levels of 200-300 pg/mL in asymptomatic individuals as a mild deficiency. Based on the threshold value of 300 pg/mL, it can be stated that 96.3% of the pregnant women (and 80% of the newborns) in our study had VB12 deficiency. Our findings were consistent with previous reports of VB12 insufficiency in Türkiye.[16,17]

Kalay et al. reported a negative correlation between neonatal VB12 levels and the number of births and birth weight.[17] We also observed a decrease in cord blood VB12 levels as the number of pregnancies increased. Similar to

our findings, a Norwegian study also noted a decline in neonatal VB12 levels with increased parity, in addition to a significant correlation between time since last birth and cord blood VB12 levels.[22] However, our study did not demonstrate an influence of period since last birth on cord blood VB12 levels.

The percentage of vaginal delivery was statistically higher in mothers with sufficient VB12 stores in our study. Zanardo et al. reported higher homocysteine concentrations in women undergoing elective C-sections under general anesthesia as well as in their babies.[23] Homocysteine elevation might result from maternal VB12 deficiency, but the study did not assess VB12 status, and the serum samples were collected after delivery. The authors assumed hyperhomocysteinemia to be caused by labor-induced hormonal changes and/or pharmacological interventions. The Turkish Ministry of Health strongly encourages vaginal birth and does not approve C-sections unless medically necessary. If C-sections were supposedly due to medical emergencies, and vaginal deliveries might have indicated a healthier pregnancy for both the mother and the fetus, one might assume that better prenatal care led to higher VB12 levels. In the current literature, maternal VB12 deficiency is linked to an increased risk of common pregnancy complications with adverse perinatal outcomes.[24] The reasons for C-sections were not investigated in our study. As a result, the reason for this correlation is unclear based on our current knowledge.

Mothers who received antenatal iron/multivitamin supplements had higher levels of VB12. However, irrespective of the supplementation, their mean VB12 levels remained below 200 pg/mL, and the difference was not reflected in maternal homocysteine, cord blood VB12, or homocysteine levels. Maternal VB12 levels directly correlate with neonatal VB12 stores, with an impact on neurological development. However, the effect of VB12 supplementation during pregnancy on offspring postpartum growth and neurodevelopment is still unknown. Srinivasan et al. showed that maternal VB12 supplementation during pregnancy had no effect on cognitive development in infants at 9 months of age, but higher maternal homocysteine levels were associated with poorer cognitive performance in some Bayley Scales of Infant Development-III subdomains.[25] Chandyo et al. demonstrated an improvement in maternal VB12 status but no improvement in infant growth and development in a double-blind randomized trial.[26]

Because of hemodilution and decreased haptocorrin production during pregnancy, serum VB12 concentrations fall, rendering this test inaccurate.[10] Hyperhomocysteinemia has been proposed as a measure of VB12 insufficiency in tissues, with controversial utility in diagnosing VB12 deficiency.[8] Despite the high prevalence of VB12 deficiency in our study, only about 26% of pregnant women exhibited a substantial increase in homocysteine levels, and none of them had evidence of VB12 deficiency-related signs or symptoms. This finding suggests that homocysteine alone is not always a reliable indicator of VB12 deficiency nor a suitable screening test for diagnosis, as Amarasinghe et al. previously reported.[27] Since serum VB12 assays estimate total VB12 rather than directly indicating metabolic utilization, it is challenging to definitively diagnose VB12 deficiency based on serum levels alone. There are no universally accepted cut-off values for holotranscobalamin, homocysteine, and methylmalonic acid as well.[28] Therefore, there is still a lack of consensus about the threshold value or the ideal combination of tests for diagnosing VB12 deficiency in pregnancy.

Despite the high prevalence of maternal VB12 deficiency, babies had a lower prevalence of VB12 deficiency. The same difference was observed in comparison to mean VB12 levels. In many studies from various countries, cord blood VB12 levels were found to be 27-100% higher than those of the mother.[1] In our study, this ratio was found to be 28%. This situation may be considered as a physiological response

for the baby's protection, similar to which has been reported for iron stores of babies in relation to maternal status.[29] Also, maternal VB12 measurement may not be a reliable determinant of VB12 deficiency, as circulating levels of holotrancobalamin (active VB12) might remain relatively stable during pregnancy.[9]

The relationship between VB12 status, birth weight, and gender remains controversial. Tanyildiz et al. reported lower birth weights in children with VB12 deficiency born to VB12 deficient mothers in comparison to those born to VB12 sufficient mothers. Conversely, a Norwegian study found lower cobalamin levels in heavier neonates and female neonates[22,30], our study identified a negative correlation between infants' birth weights and cord blood VB12 levels. However, it was not considered clinically important because the difference between the mean weights of newborns with and without VB12 deficiency was 67 g. There was no significant difference in the VB12 levels of male and female neonates' cord blood as well.

In our study, in babies of mothers with VB12 levels below 200 pg/mL, VB12 levels were significantly lower and homocysteine levels were significantly higher. These results are similar to findings reported from our country as well as developed countries[1,21,31] who recommend VB12 supplementation during pregnancy and lactation.[21]

The sample size and number of parameters evaluated were the study's strengths. The limitation of our study is the lack of assessment of nutrition and serum iron and folate status in mothers, as serum levels of homocysteine might also be influenced by folic acid, Vitamin B6, and betaine.

The different cut-off values and tests to diagnose VB12 deficiency, diverse maternal dietary habits, gastrointestinal risk factors, mode of birth, antenatal vitamin supplementation, and gestational age are the challenges to making a healthy comparison among studies on VB12 deficiency. As a result, each country may require a distinct approach to assessing VB12 status based on epidemiological studies and its specific socioeconomic and healthcare system. Our study revealed a significant VB12 deficiency in pregnant women and their babies, highlighting a need for a systematic exploration of health issues associated with insufficient VB12 in pregnant women and their babies, antenatal assessment of VB12 status, and VB12 supplementation if necessary.

Ethical approval

University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital (approval number and date: 3132-2/2/2021).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ZYY, DBG, AK, VM, SS, KD; data collection: ZYY, DBG, AK, VM, SS, KD, MAC, NDE, AE, NCC, EO, EK; analysis and interpretation of results: ZYY, DBG, AK; draft manuscript preparation: ZYY, DBG. All authors revised and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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