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Original Article

Maternal and Cord Blood Vitamin B12, Folate and Homocysteine Levels

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ABSTRACT

Aim: Nowadays, insufficiency and deficiency of vitamin B12 and folate are seen as an important health problem. The purpose of the present study was to determine the frequency of vitamin B12 and folate deficiencies in pregnant women and their babies at birth.

Materials and Methods: The study group consisted of 117 pregnant women and their single, term babies in İzmir Ege Maternity Hospital. Analysis of vitamin B12, folate and homocysteine levels were performed from venous blood samples which were obtained from the mother and cord blood at birth. Additionally, a questionnaire using a face-to-face interview method was performed with the pregnant women included in this study. The mean duration of pregnancy was 39.1±0.89 weeks and the mean age of the mothers was 28.2±6.2 years.

Results: Vitamin B12 deficiency (<130 pg/mL) was present in 88.9% of mothers and 56% of babies. Folate deficiency (<4 ng/mL) was found in 6.8% of mothers, but not found in any babies. The homocysteine levels were high (>8 µmol/L) in 58.1% of mothers and 63.2% of babies. There was a significant correlation between maternal and cord blood vitamin B12, folate and homocysteine levels ($p<0.01$). However, there was no correlation between maternal vitamin B12 and homocysteine levels ($p=0.016$, $p=0.354$).

Conclusion: Low maternal vitamin B12 levels are strongly associated with low cord blood vitamin B12 levels. This data reveals that vitamin B12 deficiency which can occur from the neonatal period is a preventable public health problem. Pregnant women and physicians should be made aware of the importance of vitamin B12 intake during pregnancy.

Keywords: Folate, homocysteine, vitamin B12

Introduction

Pregnancy is a period of rapid development for both the mother and the fetus and the requirement for some nutrients increases during pregnancy (1). Almost 20% percent of females have vitamin B12 and folate deficiency (2). Vitamin B12 is a water-soluble, red colored vitamin that is essentially synthesized by microorganisms at the end of nearly twenty different enzymatic stages. Inadequate dietary intake of vitamin B12 is a fundamental reason for cobalamin deficiency because the vitamin B12 that is required for humans is totally supplied by the diet from animal food. The other causes of vitamin B12 deficiency are autoimmune diseases, environmental pollution, malabsorption and non-separating of cobalamin bound to food (3). Vitamin B12 is an essential cofactor of enzymes which play a role in the synthesis of DNA, fatty acids and myelin. For this reason, symptoms of hematological, neurological diseases and neuropsychiatric diseases may occur. Vitamin B12

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deficiency predisposes neurodevelopmental diseases and megaloblastic anemia. Folate is a form of water-soluble vitamin B9. It has different missions for different body cells, especially in purine and pyrimidine synthesis. Pregnant women have a high risk of folate deficiency because of an increased requirement and the catabolism of folate. Folate deficiency is related with ablatio placenta, spontaneous abortion, prematurity, congenital defects such as neural tube defects and perinatal mortality. The Centers for Disease Control and Prevention advices 400 µg/day folic acid replacement during the period starting from preconception until the 20th week of pregnancy (4). Both folate and vitamin B12 are essential cofactors for homocysteine metabolism (5). In addition, high levels of plasma homocysteine are associated with vascular endothelial damage and occlusive vascular problems. Therefore, this situation poses a high risk for renal and cardiovascular diseases. Increased plasma homocysteine concentration is related with vascular placental thrombosis and may cause preeclampsia and ablasio placenta which result in early pregnancy losses (6).

The aim of this study was to evaluate the ratio of maternal vitamin B12 and folate deficiency and to analyze maternal and cord blood vitamin B12, folate and homocysteine levels. For this reason, we evaluated the relationships between maternal and cord blood vitamin B12 and folate status.

Materials and Methods

Study Population

Those pregnant women who met the inclusion criteria and agreed to take part in this study were included in this study. This study took place during the month of December 2012 in İzmir Ege Maternity Hospital. The inclusion criteria for the study were having a single, term birth (38-42 weeks). Preterm birth (≤37 week), birth after the 42nd week and multiple births were exclusion criteria. Both normal and cesarean deliveries were included. Written consent was received from the pregnant women who took part in this study. The sample size was calculated to be 97 with 95% confidence interval.

Study Design and Ethical Approval

This retrospective cross-sectional study was approved by the Clinical Research Ethics Committee of Ege University, Faculty of Medicine Hospital on 19.10.2012 (decision no: 12-9.1/10).

Data Collection

The study group consisted of 117 pregnant women and their single, term babies in İzmir Ege Maternity Hospital. A questionnaire via a face-to-face interview method was performed with the pregnant women. Socio-demographic features (mother's age, economic status, mother and father's education and profession, nutritional status during pregnancy, usage of vitamin/mineral supplements, maternal illness during pregnancy, use of cigarettes, alcohol or substances during pregnancy) were investigated. Birth weight-height, gender, gestational age, and congenital anomaly presence were recorded at birth. Analysis of vitamin B12, folate and homocysteine levels were carried out on venous blood samples which were obtained from the mother and the cord at birth. The blood samples were protected by centrifugation at -20 °C degree until analysis. A chemiluminescence immunoassay technique with Immulite 2000 was used for analysis.

Data Analysis

One hundred thirty pg/mL was accepted as the deficiency limit for serum B12 vitamin levels of both the pregnant women and their babies. Measured serum cobalamin levels do not reflect metabolic active vitamin B12 status exactly. There is no correlation between the occurrence of clinical findings and serum vitamin B12 levels. The lowest level seen in studies was taken as the limit for serum vitamin B12 levels as there is no clear cut-off limit in the literature. Four ng/mL has been determined as the deficiency level of folate according to the criteria of NHANES 3 classification in 2005 (7). Homocysteine is used as a metabolic indicator for this classification. Hyperhomocysteinemia during pregnancy is defined as ≥8 µmol/L based on the literature (8). A hemoglobin value below 11 gr/dL is accepted as the anemia level for pregnant women.

Statistical Analysis

Data was evaluated using the SPSS 18.0 (Statistical Package for Social Sciences) statistical package software. Categorical variables were expressed as number (n) and percentage (%). Comparisons between groups of qualitative variables were determined using the chi-square test, numerical variables were compared using Student's t-test and One-Way ANOVA. Multiple logistic regression analysis was used to investigate potential independent risk factors which may affect vitamin B12, folate or homocysteine levels. Correlation analysis was used to determine the relationship between numerical variables such as the maternal and cord blood vitamin B12, folate and homocysteine levels. Statistically significance was considered for $p<0.05$.

Results

During the one month period of the study, there were totally 177 births. Six births were multiple pregnancy, 19 births were <37 weeks and 5 births were >42 weeks, 24 pregnant women did not agree to participate in this study and 6 of the samples were insufficient. As a result, the study was carried out with 117 pregnant women and their babies. The average gestational week was 39.1±0.89. Fifty-three percent of babies were male. The mean birth weight was 3,402±47.5 gr, and the mean birth height was 50.24±1.22 cm. There was no congenital anomaly in 97.4% of babies. Two babies had antenatal bilateral hydronephrosis and one baby had antenatal sacral teratoma pre-diagnosis. The socio-demographic and some laboratory characteristics of the mothers and babies are given in Table I and the descriptive characteristics of pregnant women are given in Table II.

According to the classifications of the education status of the mothers, 59.8% of the mothers had primary school level or lower and 9.3% of them had high school or higher educational status. There was no vitamin/mineral supplementation for 12.8% of mothers, 52.9% of them had 3 months or less, 32.4% of them had between 3-6 months, and 14.7% of them had more than 6 months vitamin/mineral supplementation. However, detailed information about the name of the preparation and its contents could not be obtained from those pregnant women who stated that they had taken vitamin/mineral supplementation during pregnancy.

The nutritional habits of the mothers during their pregnancy were investigated. It was observed that 2.6% of them had a vegetarian diet and 83.8% of them consumed red meat every 2 weeks or less (Table III).

The mean maternal and cord blood vitamin B12 levels were determined to be 73±40.6 pg/mL and 121.7±68.36 pg/mL respectively in this study.

The maternal and cord blood B12 deficiency rates were determined to be 88.9% and 59% respectively. The mean maternal and cord blood folate levels were 12.43±7.31 ng/mL and 22.2±4.39 ng/mL respectively in our study. The maternal folate deficiency rate was 6.8%, but all of the babies had normal folate levels. It was found that the babies of those pregnant women with low serum vitamin B12 levels had more vitamin B12 deficiency (p<0.01) (Table IV). When the limit for hyperhomocysteinemia is accepted as 8 µmol/L; high serum homocysteine levels were seen in 58.1% of the mothers, while this ratio was 63.2% in cord blood. The mean maternal and cord blood homocysteine levels were 8.98±3.2 µmol/L and 9.2±3.03 µmol/L respectively.

When the relationship between serum vitamin B12 deficiency and hyperhomocysteinemia was examined, no statistically significant difference was found for maternal serum homocysteine levels between those mothers with vitamin B12 deficiency and those without (p>0.05). Homocysteine levels were high in 59.6% of those mothers with low serum vitamin B12 levels and this ratio was 46.2% for those mothers who had normal vitamin B12 levels. The relationship with folate deficiency, which is one of the reason of hyperhomocysteinemia, was also examined. Fifty percent of the 8 mothers with low serum folate levels had normal serum homocysteine levels. Ninety-four percent of the 68 mothers who had high serum homocysteine levels had normal serum folate levels (p>0.05). Gender was not related with vitamin B12 and folate levels, however homocysteine levels were statistically higher for the male babies (p=0.026) (Table IV). There was no statistical

Table I. Socio-demographic and laboratory characteristics of mothers and babies

	Mean	Standard deviation	Minimum value	Maximum value
Birth weigth (gr)	3402.0	474.5	1750	4500
Birth height (cm)	50.24	1.22	47	53
Gestational age (week)	39.15	0.89	38	42
Mother age (year)	28.2	6.2	18	42
Maternal folate (ng/mL)	12.43	7.31	2.00	25.5
Maternal vitamin B12 (pg/mL)	73.0	40.6	30.00	208.0
Maternal homocysteine (µmol/L)	8.98	3.2	1.32	21.6
Cord blood folate (ng/mL)	22.2	4.39	8.1	25.0
Cord blood vitamin B12 (pg/mL)	121.7	68.36	30.0	339.0
Cord blood homocysteine (µmol/L)	9.2	3.03	4.21	19.14

difference between cord blood vitamin B12 deficiency and homocysteine levels. All babies had normal cord blood folate levels so its relation with homocysteine levels was not analyzed. The relation between the concentration of maternal and cord blood vitamin B12, folate and homocysteine was analyzed by Spearman correlation analysis (Table V). There was a strong relationship between maternal and cord blood folate levels (p=0.306, p<0.01), between maternal and cord blood vitamin B12 levels (p=0.499, p<0.01) and between maternal and cord blood homocysteine levels (p=0.483, p<0.01). There was also a strong correlation between maternal vitamin B12 and hemoglobin levels (p=0.53; p<0.01). The relation between the concentration of maternal and cord blood vitamin B12, folate and homocysteine were analyzed with Spearman correlation analysis. There was a strong relationship between maternal and cord blood folate levels (p=0.306, p<0.01), between maternal and cord blood vitamin B12 levels (p=0.499, p<0.01) and between maternal and cord blood homocysteine levels (p=0.483, p<0.01). There was also a strong correlation between maternal vitamin B12 and hemoglobin levels (p=0.53, p<0.01). There was a negative correlation between cord blood vitamin B12 and homocysteine levels (p=-0.236, p<0.05).

Anemia was found in the analysis of 38.8% of mothers with vitamin B12 deficiency and 7.7% of mothers with normal vitamin B12 levels (p<0.05). When we examined relationship between maternal folate levels and anemia, 85.7% of mothers who had folate deficiency had anemia (p<0.01) but 31.7% of mothers had anemia despite having normal serum folate levels. There was no relation between the maternal educational status and maternal vitamin B12 or folate levels (respectively p=0.082, 0.325). It was determined that there was no statistical difference between anemia in pregnant women and the number of parity, maternal educational status, maternal smoking during pregnancy or the economic status of the families. However, it was found that the frequency of anemia was lower in those mothers who used vitamin-mineral supplementation during pregnancy (p<0.01). None of the independent variables had a meaningful effect on the dependent variable when independent variables were defined as the parity number of mothers, vitamin/mineral supplementation during pregnancy, maternal education status, or maternal smoking in pregnancy and the dependent variable was defined as serum vitamin B12. Smoking, which is one of the reasons of hyperhomocysteinemia, was not related with maternal and cord blood homocysteine levels in this study.

Table II. Descriptive characteristics of pregnant women

Characteristics	Number (n)	%
Parity of mother		
1	42	35.9
2	44	37.6
≥3	31	26.5
Education status of mother		
Uneducated	17	14.5
Primary school	53	45.3
Middle school	35	29.9
High school and upper	12	9.3
Vitamin/mineral supplements during pregnancy		
Yes	102	87.2
No	15	12.8
Smoking during pregnancy		
Yes	26	22.2
No	91	77.8
Alcohol consumption during pregnancy		
Yes	0	0
No	117	100

Table III. Analysis of mothers' nutrition during their pregnancy

Consumption frequency	Red meat (%)	Poultry (%)	Fish (%)	Egg (%)	Milk (%)	Dairy product (%)	Green vegetables (%)
Daily	0.9	0.9	0.0	41.0	27.4	89.7	82.1
4-5 times/week	1.7	1.7	1.7	19.7	7.7	6.0	6.8
1-2 times/week	13.7	59.8	47.9	19.7	18.8	3.4	9.4
1 times/2 weeks	36.8	19.7	13.7	4.3	1.7	0.0	0.0
1 times/month	39.3	10.3	19.7	0.9	1.7	0.0	0.0
Never	7.7	7.7	17.1	14.5	39.3	0.9	0.9

Discussion

In this study, it was shown that the babies of pregnant women with low serum vitamin B12 levels had more severe vitamin B12 deficiency ($p<0.01$) and there was a strong correlation between maternal and cord blood vitamin B12 levels ($p=0.499$, $p<0.01$). Studies conducted with similar population groups in developing countries and our country support the view that the vitamin B12 levels of mothers and their newborns are very closely correlated and show that vitamin B12 deficiency is severely high in mothers and infants. In a study carried out in Brazil, a developing country like our country, blood samples of 69 pregnant women and their babies were examined at birth in 2002 (9). The mean serum maternal vitamin B12 levels were 154.1±77.8 pmol/L (208.6±104.3 pg/mL), these levels were determined to be 256.8±198.9 pmol/L (346.8±268 pg/mL) for the babies. There was a strong correlation between maternal and neonatal B12 levels ($r=0.68$, $p<0.01$). Similar to our results, cord blood vitamin B12 levels were found to be 2-3 times higher than the maternal values. The vitamin B12 deficiency ratio in pregnant women was determined to be 74.1% in a study that was designed by Pathak et al. (10) in India. Vitamin B12 deficiency was found in early pregnancy (at 13-17 gestational weeks) to be 48.8%, in later pregnancy (at 28-32 gestational weeks) to be 80.9% and at postpartum 13-17 weeks to be 60%. Açkurt et al. (11) designed their study with prenatal and postnatal nutritional evaluation of 133 randomized pregnant women in our country, in Istanbul and Izmit. Onal et al. (12), evaluated 250 mothers and their term babies at postnatal 48^{th} hour in 2010. The lowest limit of vitamin B12 was accepted to be 300 pg/mL for mothers and 200 pg/mL for babies in this study. Vitamin B12 deficiency was found in 81.6% of mothers and 41% of their babies. In a similar study which was designed in the northern region of Turkey between 2008 and 2009, the ratio of maternal vitamin B12 deficiency was 72% and this ratio was 56% for their babies (13). In another study in Urfa, values below 160 pg/mL were defined as deficiency and values below 120 pg/mL were defined as severe deficiency for both mothers and babies (14). The vitamin B12 deficiency ratio was 72% (severe deficiency 48%) for mothers and 41% for babies (severe deficiency 23%) (14). There was also a statistically significant correlation between maternal and cord blood vitamin B12 levels ($r=0.395$, $p<0.001$). In a study conducted with 72 pregnant women at the same time and in a similar region of Turkey as our study, it was observed that 70.8% of mothers and 83.9% of infants had vitamin B12 deficiency when 200 pg/mL was accepted as the cut-off point for vitamin B12 (15). It is considered that the dominance of the Mediterranean diet in the region might be responsible.

There is no clear limit value for defining serum vitamin B12 deficiency in the literature. Different reference ranges have been determined according to the methods and kits which are used. RDA reports the lowest limit of vitamin

Table IV. Relationship between maternal serum vitamin B12, folate levels, gender and maternal serum homocysteine level

	Cord blood vitamin B12 level		
	Low	**Normal**	**p-value**
Maternal vitamin B12 level			
Low, n (%)	66 (63.5)	38 (36.5)	0.005
Normal, n (%)	3 (23.1)	10 (76.9)	
	Maternal homocysteine level		
	Normal	**High**	**p-value**
Maternal vitamin B12 level			
Low, n (%)	42 (40.4)	62 (59.6)	0.354
Normal, n (%)	7 (53.8)	6 (46.2)	
Maternal folate level			
Low, n (%)	4 (50)	4 (50)	0.63
Normal, n (%)	45 (41.4)	64 (58.7)	
Gender			
Male, n (%)	17 (27.4)	45 (72.6)	0.026
Female, n (%)	26 (47.3)	29 (52.7)	

Table V. Relationship between maternal and cord blood vitamin B12, folate and homocysteine concentrations (Spearman correlation analysis)

	Cord folate	Cord B12	Cord Hcy	Maternal Hcy	Hb
Maternal folate	0.306**	0.152	-0.168	-0.126	0.164
Maternal B12	0.101	0.499**	-0.119	-0.016	0.53**
Maternal Hcy	-0.129	-0.105	0.483**		-0.014
Cord Hcy	-0.098	-0.236*		0.483**	

*p<0.05, **p<0.01

B12 as 120-180 pmol/L (170-250 pg/mL) for adults (16). In addition, serum total vitamin B12 concentration starts to decrease during the early processes of the first trimester of pregnancy. This decrease becomes more significant at 6 months. For this reason, in our study, 130 pg/mL was accepted as the limit of vitamin B12 deficiency despite this value being one of the lowest values for vitamin B12 deficiency. The rates of vitamin B12 deficiency in mothers and babies were found to be slightly higher in this study. The mean vitamin B12 levels were also lower compared to similar studies. It is considered that the reason for this might be related with this study group which represented a poor socio-economic region. Our findings may be related with the mothers' nutritional insufficiencies which continue during their pregnancy and so their newborn babies were born with insufficient vitamin levels. When the nutritional status of the mothers during pregnancy was investigated, they were seen to be inadequate. Waldmann et al. (17) compared strict and mild vegetarians in their study. Strict vegetarians had significantly lower cobalamin levels and higher homocysteine levels. However, there was no difference found with folate levels.

It was observed that the number of births and pregnancies of the mother did not affect the serum and cord blood vitamin B12, folate and homocysteine levels when evaluated by logistic regression analysis. In addition, there was no relationship between whether the mother used vitamin-mineral support during pregnancy and maternal serum and cord blood vitamin B12 and folate levels. However, 65.7% of the mothers had used vitamin-mineral support for less than 3 months during pregnancy. This period is quite short and it is thought that the relationship could not be clearly determined since the content of the preparations used is not known.

Monagle and Tauro (18) determined that the most frequent reason of infantile megaloblastosis was maternal vitamin B12 deficiency and they stated that fifty percent of these mothers were asymptomatic. All of these data support the view that babies who are born to mothers who have insufficient vitamin B12 stores, substantially also have insufficient vitamin B12 stores at birth (19). Normally, babies are born with 25-50 mcg vitamin B12 storage. The essential quantity for growth is 0.1 mcg/day. This vitamin B12 store which a newborn has at birth is enough for 6-12 months. The development of infants with insufficient vitamin B12 storage is generally normal for the first months. If the initiation of complementary nutrition is delayed, clinical features may present due to vitamin B12 deficiency after sixth months (20,21). Prenatal 3rd trimester and postnatal 3-6 months is the period of life in which brain development and myelination are fastest. If the mother has insufficient vitamin B12, the baby's vitamin B12 deficiency develops earlier (19). Cerebral atrophy and hypoplasia may develop at birth because of the effects of myelinization during the last trimester. If vitamin B12 deficiency is not detected and treated early, it may result in irreversible neurological damage in infants (22-25). The most common symptoms are lethargy, hypotonia and convulsions, and sometimes encephalopathy may present. Severe pancytopenia may develop in addition to megaloblastic anemia due to vitamin B12 deficiency because of impairment of DNA synthesis. Hypogammaglobulinemia may accompany vitamin B12 deficiency and reach normal levels with vitamin B12 treatment (26). In a study with a large sample size designed in Holland in 2010, it was claimed that maternal vitamin B12 deficiency might be an etiological reason for excessive crying in babies (27). In a comparison of two groups of pregnant women in India, oral vitamin B12 (50 µgr) was given to one group during pregnancy and early lactation and a placebo were given to the other group (28). Vitamin B12 levels were statistically higher in the group taking vitamin supplements in an evaluation of the breast milk, the maternal serum and the babies' serum at postnatal 6th week. In a systematic review, the importance of vitamin B12 supplementation during pregnancy is emphasized according to results which state that having maternal vitamin B12 levels <148 pg/L is a risk factor for low birth weight for newborns and there is a linear relationship between maternal B12 vitamin levels and preterm birth (29). In addition, in a study designed as premarital screening of women, vitamin B12 and iron deficiency were seen to be an important health problem for women of reproductive age in our country (30).

There was also a significant relation between maternal and cord blood folate levels (p=0.306; p<0.01) in our study. The mean maternal folate levels were 13.9±5.6 nmol/L (5.74±2.21 ng/mL) and the mean serum folate levels for babies were 6.6±2.8 nmol/L (2.65±0.88 ng/mL) at birth in a study designed by Guerra-Shinohara et al. (9) in Brazil. All of the mothers had normal folate levels and 4% of babies had folate deficiency in Sayar et al.'s (13) study. The results of the Koc et al.'s (14) study in Urfa overlapped with our results. In their study, maternal folate deficiency was found to be 12% (severe deficiency was 9%) and serum folate levels that were below 4 ng/mL were defined as deficient, and below 2 ng/mL were defined as severely deficient. All of the babies had normal folate levels as in our study. It is considered that folic acid supplementation plays a big role in these results.

Cord blood homocysteine levels were higher in males than females in this study. There have been different results in recent studies, but generally, it is accepted that there is no relation to gender (29). There was a strong correlation between maternal and cord blood homocysteine levels (p=0.483, p<0.01) and also a negative correlation between cord blood vitamin B12 and homocysteine levels (p=-0.236, p<0.05) in this study. In the study of Guerra-Shinohara et al. (9), there was a strong correlation between maternal and neonatal homocysteine levels and a negative correlation between neonatal vitamin B12 and homocysteine levels, as in our study. This indicates that there is a weak negative correlation between neonatal folate and homocysteine levels (9).

Plasma homocysteine levels decrease physiologically in normal pregnancies. It is argued that this is a physiological adaptation (31). No significant correlation was found between maternal vitamin B12 and homocysteine levels, and it is thought that homocysteine levels are not a significant indicator in revealing vitamin B12 deficiency in pregnancy due to the physiological decrease in homocysteine levels during pregnancy. As emphasized by Wallace et al. (32), vitamin B12 is not a good marker of homocysteine levels in pregnancy. There was no significant relation between smoking during pregnancy and cord blood homocysteine levels in our study. In a study in Oslo, babies whose mothers smoked 10 or more per day had significantly higher homocysteine levels (33).

Study Limitations

The daily consumption of cigarettes was not investigated in our study, and this can be considered to be an incomplete point of our study. It was observed that the frequency of anemia was 3.9 times higher in those who did not use vitamins and minerals during pregnancy. One of the limitations of this study is that we could not fully investigate at what dose and for how long vitamin-mineral support was taken, because the pregnant women did not know the exact contents of the preparations they used. Due to the fact that the region in which this study was conducted has a low socioeconomic level, it is not possible to generalize the results to the whole society. However, our findings are important in terms of emphasizing that babies are born with vitamin deficiencies in cases of maternal nutritional deficiency.

Conclusion

Maternal vitamin B12, folate and homocysteine levels are significantly correlated with cord blood levels. This study reveals that vitamin B12 deficiency which can occur from the neonatal period is a preventable public health problem. Babies may be born with adequate vitamin B12 stores by supporting their mothers' intake of vitamin B12 during and/or before pregnancy. Pregnant women and physicians should be made aware of the importance of vitamin B12 intake during pregnancy.

Ethics

Ethics Committee Approval: This retrospective cross-sectional study was approved by Clinical Research Ethics Committee of Ege University Faculty of Medicine Hospital on 19.10.2012 (decision no: 12-9.1/10).

Informed Consent: Written consent was received from the pregnant women that included to study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.Ö.B., E.Ş., O.B.H., F.K., S.A. Design: S.Ö.B., E.Ş., O.B.H., F.K., S.A. Data Collection and/or Processing: E.Ş., Analysis or Interpretation: E.Ş., Supervision: S.Ö.B., F.K., Writing: S.Ö.B., E.Ş., O.B.H., F.K., S.A.

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