_ ΕΡΕΥΝΑ _

Μελέτη των επιπέδων της βιταμίνης Β12 στην εγκυμοσύνη

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ΠΕΡΙΛΗΨΗ

Εισαγωγή: Η ανεπάρκεια της βιταμίνης Β12 εμφανίζεται συχνά κατά τη διάρκεια της εγκυμοσύνης και έχει συσχετιστεί με αυξημένο κίνδυνο κοινών επιπλοκών της εγκυμοσύνης, συμπεριλαμβανομένης της αυτόματης αποβολής, του χαμηλού βάρους γέννησης κ.α.

Σκοπός: Σκοπός της εργασίας αυτής ήταν η μέτρηση των επιπέδων της βιταμίνης Β12 σε έγκυες γυναίκες κατά τη διάρκεια και των τριών τριμήνων και η συσχέτιση τους με αιματολογικούς παράγοντες.

Υλικά και Μέθοδοι: Στη μελέτη συμμετείχαν 145 έγκυες γυναίκες ηλικίας 31,9±5,5 ετών. Τα δείγματα αίματος συλλέχθηκαν από την Γ΄ Μαιευτική και Γυναικολογική Κλινική του Γενικού Πανεπιστημιακού Νοσοκομείου «Αττικόν». Οι αναλύσεις των αιματολογικών παραγόντων πραγματοποιήθηκαν την ίδια ημέρα της συλλογής σε αναλυτή Counter Sysmex XE-2100 (ROCHE).

Αποτελέσματα: Τα επίπεδα της βιταμίνης Β12 μειώνονται σημαντικά κατά τη διάρκεια της κύησης. Το ποσοστό των εγκύων που εμφάνισαν ανεπάρκεια βιταμίνης Β12 στο πέρας του τρίτου τριμήνου σχεδόν διπλασιάστηκε σε σχέση με το δεύτερο τρίμηνο και προσέγγισε το 47.2%, δηλαδή σχεδόν το ήμισυ των εγκύων του δείγματος μελέτης. Όσον αφορά το πρώτο και το δεύτερο τρίμηνο της κύησης, η συχνότητα εμφάνισης ανεπάρκειας βιταμίνης Β12 δε εμφάνισε καμία στατιστικά σημαντική ή οριακή συσχέτιση με τις κλινικές αιματολογικές παραμέτρους, την παρουσία σιδηροπενικής αναιμίας και σιδηροπενίας, καθώς και με την ηλικία και το ιστορικό αποβολών των εγκύων γυναικών του δείγματος. Τέλος, διαπιστώθηκε οτι και στα τρία τρίμηνα η βιταμίνη Β12 δεν αποτελεί στατιστικά σημαντικό παράγοντα στη διαμόρφωση της Hgb.

Συμπεράσματα: Από την αξιολόγηση των επιπέδων της βιταμίνης Β12 στο πλάσμα των εγκύων κατά το τρίτο τρίμηνο της εγκυμοσύνης, διαπιστώνεται ότι τα επίπεδα της Β12 πέφτουν σε σημαντικό βαθμό σταδιακά κατά την πάροδο της κύησης. Επιπλέον, στο πρώτο και στο δεύτερο τρίμηνο της εγκυμοσύνης, οι χαμηλές τιμές της βιταμίνης Β12 δεν έδειξαν στατιστικά σημαντική ή οριακή συσχέτιση με τις αιματολογικές παραμέτρους. Συμπερασματικά, ο έλεγχος των επιπέδων της βιταμίνης D θα πρέπει να πραγματοποιείται κατά την έναρξη της κύησης ώστε να λαμβάνεται η κατάλληλη θεραπεία ειδικά στις ομάδες υψηλού κινδύνου.

Λέξεις Κλειδιά: Ανεπάρκεια βιταμίνης Β12, εγκυμοσύνη, αιματολογικοί παράγοντες.

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ORIGINAL PAPER

Study of vitamin B12 levels in pregnancy

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ABSTRACT
Background: Vitamin B12 deficiency often occurs during pregnancy and has been associated with an increased risk of common pregnancy complications, including miscarriage, low birth weight, etc.
Aim: The aim of this study was to measure the levels of vitamin B12 in pregnant women during the three trimesters and their correlation with hematological factors.
Materials and Methods: The study involved 145 pregnant women aged 31.9 ± 5.5 years. Blood samples were collected from the C-Obstetrics and Gynecology Clinic of the General University Hospital «Attikon». Hematological factors analysis was performed on the same day of the collection on a Counter Sysmex XE-2100 (ROCHE) analyzer.
Results: Vitamin B12 levels decreased significantly during pregnancy. The percentage of pregnant women who developed vitamin B12 deficiency at the end of the third trimester almost doubled compared to the second trimester and approached 47.2%, i.e. almost half of the pregnant women in the study sample. Regarding the first and second trimesters of pregnancy, the incidence of vitamin B12 deficiency did not show any statistically significant or marginal correlation with clinical hematologic parameters, the presence of iron deficiency anemia and iron deficiency, the age, and the history of pregnant women. Finally, it was found that in all three trimesters vitamin B12 is not a statistically significant factor in the formation of Hgb.
Conclusions: The evaluation of the levels of vitamin B12 in the plasma of pregnant women during the third trimester of pregnancy showed that B12 levels decrease significantly during pregnancy. In addition, in the first and second trimesters of pregnancy, low levels of vitamin B12 did not show a statistically significant or marginal correlation with blood parameters. In conclusion, control of vitamin D levels should be performed at the beginning of pregnancy in order to receive appropriate treatment, especially in high-risk groups.

Key Words: Vitamin B12 deficiency, pregnancy, hematological factors.

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INTRODUCTION

Vitamin B12 deficiency is a common problem in many low- and middle-income countries, particularly in South Asia.[1] This is due to the high cost of animal-based foods, which are the primary source of vitamin B12, and cultural and religious factors that limit their consumption. Several studies have found that vitamin B12 deficiency is prevalent in women and children in these countries.[1]

Pregnancy is a period when vitamin B12 deficiency is particularly common.[2] Severe deficiency during pregnancy has been linked to harmful effects on the developing brain of the infant,[3] and it has been associated with an increased risk of common pregnancy complications, such as spontaneous abortion, low birth weight, intrauterine growth restriction, and neural tube defects.[4] Children born to women with vitamin B12 deficiency are also at an increased risk for adverse health outcomes, including developmental abnormalities and anemia.[5] On the other hand, infants born to mothers without vitamin B12 deficiency typically have sufficient vitamin B12 stores for the first few months of life, while deficiency mainly occurs after the first four months of the infant's life.[6]

Vitamin B12 deficiency can cause anemia during pregnancy, with hemoglobin levels less than 11.0 g/dL in the last trimester.[7] Anemia specifically caused by vitamin B12 deficiency occurs in 10-28% of uncomplicated pregnancies. About 20% of women experience

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a normal drop in vitamin B12 levels during pregnancy, with the lowest levels being reached in the third trimester.[8]

Fortunately, vitamin B12 deficiency during pregnancy can be prevented through dietary interventions or supplementation. Clinical studies conducted in Bangalore, India, and Bangladesh found that daily vitamin B12 supplementation during pregnancy and postpartum significantly improved maternal vitamin B12 levels and increased concentrations of vitamin B12 in both breast milk and infant plasma. [9,10]

Vitamin B12 deficiency is a common problem in many low- and middle-income countries, particularly in pregnant women and children. Severe vitamin B12 deficiency during pregnancy can have harmful effects on the developing fetus and increase the risk of common pregnancy complications. Children born to mothers with vitamin B12 deficiency are also at an increased risk of adverse health outcomes. However, vitamin B12 deficiency can be prevented through dietary interventions or supplementation, particularly during pregnancy and postpartum.

Materials-Methods

145 pregnant women aged 31.9±5.5 years (minimum age 15 years and maximum 46 years) participated in the study. Participants had 1.9±1.1 pregnancies with a minimum of no pregnancy and a maximum of 9 pregnancies and 0.14±0.43 miscarriages with a minimum of no miscarriage and a maximum of 2 miscarriages.

The blood samples were collected from the 3rd Obstetrics and Gynecology Clinic of the General University Hospital "Attikon" and were approved by the Bioethics Committee of the National Kapodistrian University of Athens. The present study is in accordance with the provisions of the World Medical Organization (52nd WMA General Assembly, Edinburgh, Scotland, 2000). All participant details are kept strictly confidential. The participants were informed about the purpose of this study and the confidentiality of their information and agreed to voluntarily take part in this study with their signed informed consent. Pregnant women were screened and results were recorded for the following hematological factors: hemoglobin (Hgb), hematocrit (HCT), mean erythrocyte volume (MCV), mean hemoglobin content (MCH), mean hemoglobin concentration (MCHC), erythrocyte size distribution red blood cells (RDW), ferritin and vitamin B12. Measurements were made during the first (11th-12th week), second (22nd -23rd week) and third trimester (32nd-33rd week) of pregnancy.

Blood samples for red blood cell markers (Hgb, HCT, MCV, MCH, MCHC, RDW) were collected and processed by standard laboratory procedures, using approximately 3

ml of venous blood sample, which was collected with a sterile syringe and immediately transferred to sterile vials of heparin with ethylenediaminetetraacetic acid (EDTA). Blood analyzes were performed on the same day in a Counter Sysmex XE-2100 analyzer (ROCHE). A 5 ml venous blood sample was also collected for measurement of serum ferritin and vitamin B12 in a 5 ml WEGO vial. Assays and results were analyzed with a Modular automated analyzer (ROCHE). Standard operating procedures were strictly followed in order to maintain the quality of the laboratory results. The results of hematological markers were recorded in an excel file along with other parameters such as age, number of pregnancies, abortions, last menstrual period, chronic conditions (eg malabsorption syndrome affecting iron absorption) and values of hematological biomarkers. Finally, a complete history was taken from the pregnant women, before the first blood draw..

Results

Plasma levels of vitamin B12 by trimester of pregnancy

The mean value of serum vitamin B12 levels of pregnant women at the end of the first trimester was 315±138.1pg/ml while at the end of the second trimester of pregnancy, a statistically significant decrease was observed compared to the first trimester in the mean value of the levels of serum vitamin B12 which was 268.5±94.1pg/ml (Figure 1, $p<0.0001$). Furthermore, at the end of the third trimester of pregnancy, a statistically significant decrease in the mean value of serum vitamin B12 levels (231.0±1.04pg/ml) was again observed in relation to both the first trimester (Figure 1, $p<0.0001$) and in the second trimester (Figure 1, $p<0.0001$). In summary, vitamin B12 levels drop significantly from the first to the second trimester, as well as from the second to the third trimester of pregnancy.

Incidence of vitamin B12 deficiency among the three trimesters of pregnancy

Based on the recommendations of the World Health Organization, vitamin B12 deficiency was defined as a serum vitamin B12 concentration of less than 200 pg/ml. Based on this suggested threshold, 9.1% of pregnant women developed vitamin B12 deficiency at the end of the first trimester (Figure 2). A significantly higher percentage of 26.3% of pregnant women showed vitamin B12 deficiency at the end of the second trimester (Figure 2), while the percentage of pregnant women who showed vitamin B12 deficiency at the end of the third trimester almost doubled to 47.2%, i.e. almost half of the pregnant women in the study sample (Figure 2).

Correlation of incidence of vitamin 12 deficiency with haematological parameters, anemia of pregnancy, iron

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deficiency anaemia, iron deficiency, history of miscarriage and age

The incidence of vitamin B12 deficiency, as measured and assessed at the end of the third trimester, did not show any statistically significant or marginal correlation with the age (p=0.7359) of the pregnant women in the study and their history of miscarriage (p=0.8946).

In addition, the incidence of vitamin B12 deficiency at the end of the third trimester did not show any significant or marginal correlation with serum ferritin levels (p=0.7839) and therefore was not associated with the presence of underlying iron deficiency.

Also, the incidence of vitamin B12 deficiency at the end of the third trimester was higher in pregnant women with lower HCT and Hgb values, however without statistical significance (p=0.1034 and p=0.1194, respectively). Also, in pregnant women diagnosed with iron deficiency anemia, the incidence of vitamin B12 deficiency at the end of the third trimester was higher with marginal statistical significance (p=0.0736).

Regarding the first and second trimesters of pregnancy, the incidence of vitamin B12 deficiency showed no statistically significant or marginal correlation with clinical hematological parameters, the presence of iron deficiency anemia and iron deficiency, as well as with the age and history of miscarriage of the pregnant women of the sample.

Correlation of vitamin B12 levels with hematological markers

No statistically significant differences were found between pregnant women who had normal levels of vitamin B12 compared to those who had low levels. Pregnant women who had normal vitamin B12 levels in the second trimester had significantly higher vitamin B12, MCHC, MCH, and lower platelet (PLT), red blood cell (RBC) count, and RDW levels in the first trimester and significantly higher MCH and MCV levels in the second trimester compared to pregnant women who had low levels. In addition, pregnant women with normal third-trimester vitamin B12 levels had significantly higher first-trimester vitamin B12 and MCHC, second-trimester vitamin B12, and third-trimester Hgb, MCH, and MCV (Table 1).

Evaluating the effect of B12 on hemoglobin (Hgb) levels

After the end of the first trimester, 9 women presented vitamin B12 levels lower than 190 (12.5%) while only one lower than 145 (1.3%). Accordingly, after the end of the second trimester, 26 pregnant women presented vitamin B12 levels lower than 190 (22.6%) while only 7 lower than 145 (6.08%). Finally, during the third trimester, 52 pregnant women had vitamin B12 levels

lower than 190 (38.2%) and 13 lower than 145 (9.55%). From the analyzes of the results carried out by the method of both linear (Figures 3A, 4A, 5A) and logistic (Figures 3B, 4B, 5B) regression, it was found that in all three trimesters vitamin B12 is not a statistically significant factor in Hgb formation.

DISCUSSION

From our study, were evaluated the plasma levels of vitamin B12 in pregnant women during the third trimester of pregnancy and found that the levels of vitamin B12 gradually decreased over time from the first to the third trimester. Pregnant women who had normal vitamin B12 values at the beginning of pregnancy had significantly higher levels of vitamin B12 in all three trimesters, compared to those who had lower values at the beginning of pregnancy. These results are consistent with those of another study that found a high prevalence (50-70%) of vitamin B12 deficiency in western and southern regions of India, with lower vitamin B12 values in the third trimester of pregnancy compared to those in the early stages of pregnancy. [11]

Our study also found that pregnant women who had better vitamin B12 values in the 11th week of pregnancy had normal vitamin B12 levels in the 22nd week compared to those who had lower values in the 11th week. Similarly, pregnant women who had normal levels of vitamin B12 at 33 weeks of gestation had a better value profile at both 22 and 11 weeks compared to those who had low levels at 11 weeks of gestation.[1] Overall, 85% of pregnant women had normal vitamin B12 levels at the start of pregnancy, but this dropped to 58% at 33 weeks.

In addition, our study found that women with normal vitamin B12 values at 33 weeks of gestation had a better profile of hematological markers (MCH, MCHC and HGB) from 11 weeks of gestation compared to those with lower values in the 11th week. [1] However, low values of vitamin B12 did not show a statistically significant or marginal correlation with hematological parameters in the first and second trimesters of pregnancy.

As vegetarianism and veganism become increasingly popular, there is growing concern about the increasing incidence of vitamin B12 deficiency.[12] The Food Standards Agency's two-year "Food and You" survey in the UK found that the proportion of people who reported never consuming dairy products had increased from 2% to 5% between 2012 and 2018. Vitamin B12 deficiency can be difficult to detect as the effects may be delayed, and the body's stores of vitamin B12 may last for years.[12]

The World Health Organization (WHO) and the US National Institutes of Health (NIH) recommend a higher daily dose of vitamin

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B12 in pregnant women than in non-pregnant women to support fetal neurological development. Treatment of vitamin B12 deficiency in pregnancy is similar to that outside of pregnancy and can be achieved by oral or parenteral replacement.[13] When using oral vitamin B12 1000 μg daily, serum levels should be monitored to ensure adequate replenishment. Women at high risk or with a known deficiency should take vitamin B12 supplements during pregnancy or while breastfeeding.[14]

A study suggests that national policymakers should consider introducing screening for vitamin B12 deficiency in antenatal and neonatal screening programs to determine whether mothers and infants are at risk of vitamin B12 deficiency.[12] There are currently no published guidelines for prenatal diagnosis and management of vitamin B12 deficiency, which highlights the need for further research in this area.

In summary, our study found that vitamin B12 levels gradually decrease over time from the first to the third trimester of pregnancy. Pregnant women with normal vitamin B12 values at the beginning of pregnancy had higher levels of vitamin B12 in all three trimesters. Low values of vitamin B12 in the first and second trimesters of pregnancy did not show a statistically significant or marginal correlation with hematological parameters. Vitamin B12 supplementation during pregnancy and breastfeeding is recommended for women at high risk or with a known deficiency. There is a need for further research and guidelines for the prenatal diagnosis and management of vitamin B12 deficiency, particularly for high-risk populations. National policymakers should consider introducing screening for vitamin B12 deficiency in antenatal and neonatal screening programs to identify at-risk mothers and infants.

In addition, as vegetarianism and veganism become more common, healthcare providers should educate women about the importance of adequate vitamin B12 intake during pregnancy and breastfeeding. A balanced and varied diet that includes sources of vitamin B12, such as meat, fish, and dairy products, can help prevent vitamin B12 deficiency. For those who follow a plant-based diet, fortified foods or supplements may be necessary to meet vitamin B12 requirements.[12]

In conclusion, our study adds to the growing body of evidence highlighting the importance of vitamin B12 levels during pregnancy. Low vitamin B12 levels during pregnancy may have adverse effects on the developing fetus and maternal health. Pregnant women should be screened for vitamin B12 deficiency and receive appropriate supplementation or treatment as needed. Adequate education and dietary counseling can also help prevent

vitamin B12 deficiency in high-risk populations.

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ANNEX

FIGURE 1. Comparative study of vitamin B12 levels between the three trimesters of pregnancy

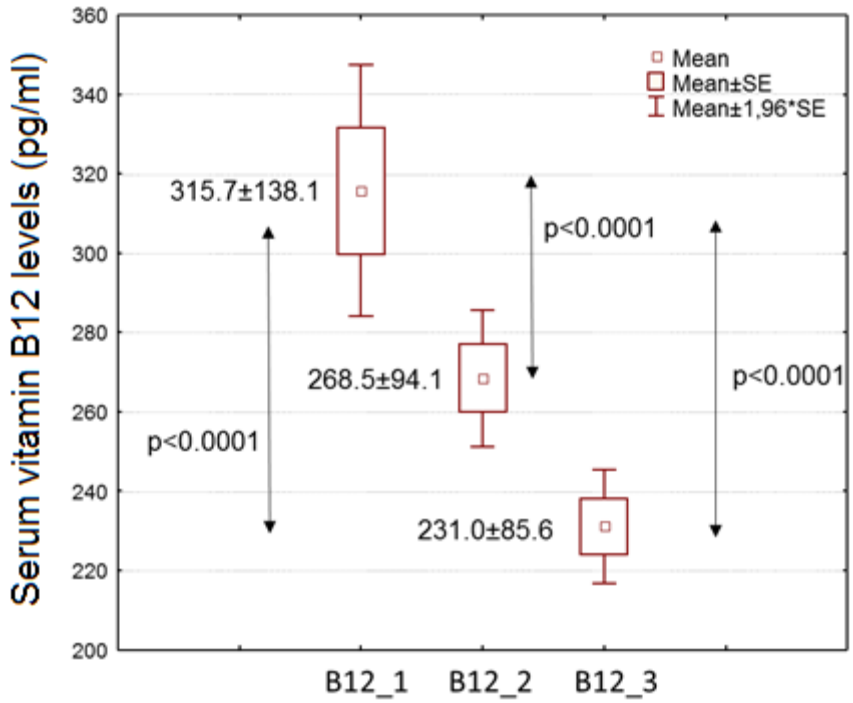

FIGURE 2. Frequency of vitamin B12 deficiency in the three trimesters of pregnancy

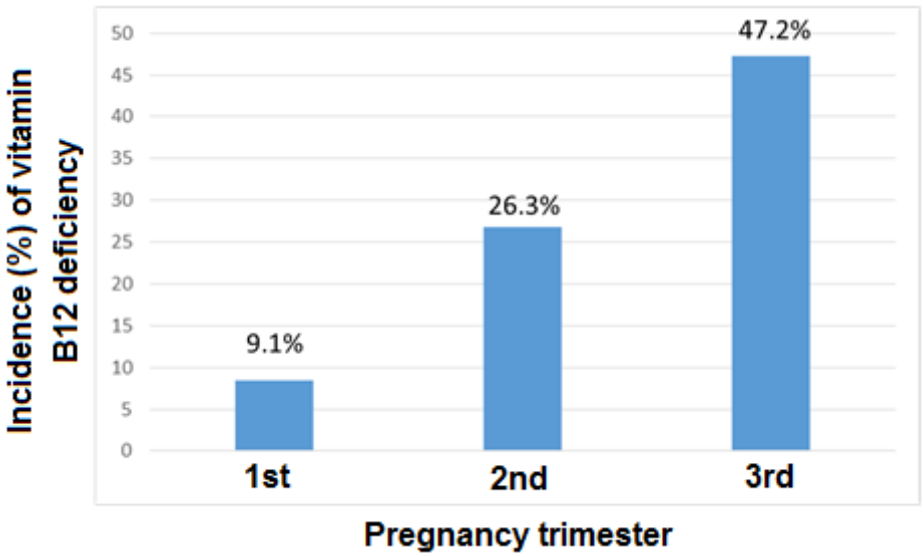

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TABLE 1. Associations between pregnant women with normal versus low levels of vitamin B12 in the second and third trimesters of pregnancy.

Hematological Parameters	**Normal 2nd trimester levels (N=85)**	**2nd trimester lows (N=16)**	**P**
B12 (pg/mL) 1st trimester	355.50±127.09	212.79±70375	<0.001
MCHC (g/dL) 1st trimester	32.82±1.71	32.07±1.86	0.046
MCH (pg) 1st trimester	29.36±2.48	27.78±3.70	0.010
PLT (K/μl) 1st trimester	244.76±58.03	272.47±74.03	0.039
RBC (10/L) 1st trimester	4.25±0.37	4.45±0.47	0.023
RDW (%)1st trimester	13.79±1.61	14.66±1.94	0.017
MCH (pg) 2nd trimester	29.99±2.65	28.37±3.67	0.011
MCV (fL) 2nd trimester	90.17±8.21	85.04±8.29	0.004
Hematological Parameters	**Normal 3rd trimester levels (N=79)**	**3rd trimester lows (N=58)**	**P**
B12 (pg/mL) 1st trimester	367.46±142.29	240.60±79.36	<0.001
MCHC (g/dL) 1st trimester	32.73±1.89	32.06±1.86	0.043
B12 (pg/mL) 2ο τρίμηνο	316.12±84.67	199.81±55.83	<0.001
HGB (g/dL) 3rd trimester	11.59±1.05	11.24±0.99	0.049
MCH (pg) 3rd trimester	29.99±2.77	28.74±3.73	0.025
MCV (fL) 3rd trimester	88.33±7.60	85.57±8.53	0.049

FIGURE 3. Scatter plot between vitamin B12 and Hgb after the end of the 1st trimester by the method of linear regression (p-value: 0.636, Pearson's correlation coefficient: -0.0571 (A)) and logistic regression (p-value: 0.605) (B).

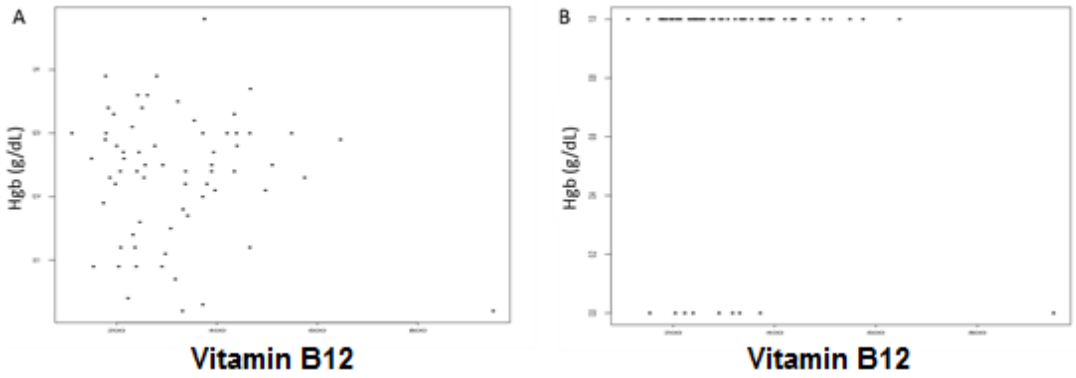


Figure 4. Scatter plot between vitamin B12 and Hgb after the end of the 2nd trimester by the method of linear regression (p-value: 0.36, Pearson's correlation coefficient: 0.0861 (A)) and logistic regression (p-value: 0.803) (B).

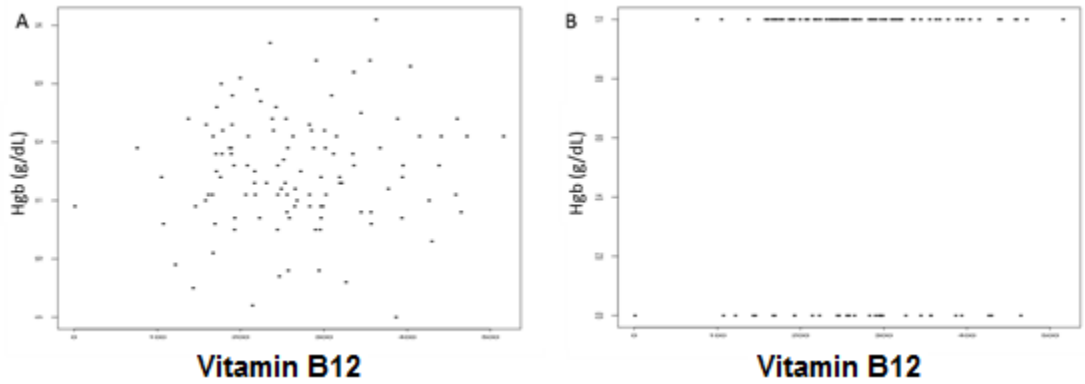
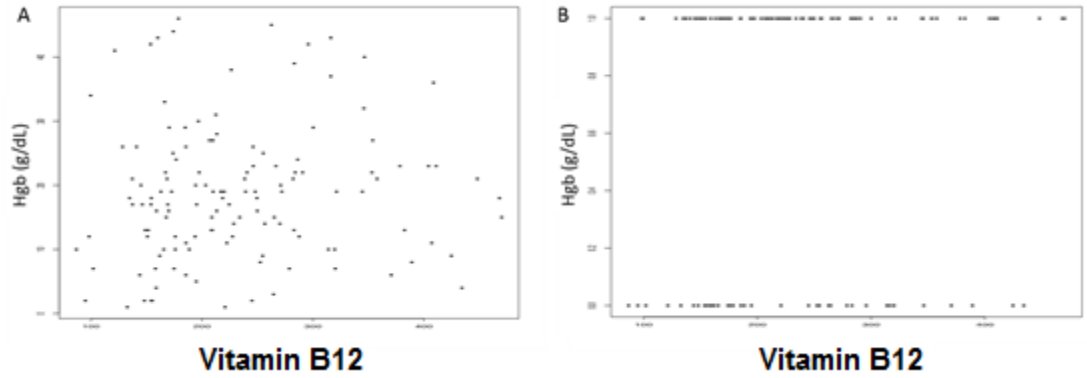


Figure 5. Scatter plot between vitamin B12 and Hgb after the end of the 3rd trimester by the method of linear regression (p-value: 0.402, Pearson's correlation coefficient: 0.0729 (A)) and logistic regression (p-value: 0.453) (B).



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