ORJİNAL ÇALIŞMA

TÜRK GEBE POPÜLASYONUNDA 1.2.3. TRİMESTER'DEKİ VIT B12, FOLAT VE HOMOSİSTEİN DÜZEYLERİNİN DEĞERLENDİRİLMESİ

Vitamin B12, Folate and Homocystein Status in Turkish Pregnant Women for each Trimester

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ÖZET

Amaç: Vitamin B12 ve folat, hücre büyümesi ve farklılaşması için esansiyel mikronutrientlerdir. Bu kesitsel çalışmadaki amacımız, vitamin desteği almayan sağlıklı Türk gebe kadınlardaki vitamin B12 ve folat ortalamalarını ve eksiklik prevelanslarını tespit etmektir.
Method: Bu prospektif kesitsel çalışma Ocak 2016- Mayıs 2017 tarihleri arasında gerçekleşmiştir. Birinci trimesterdeki, vitamin desteği almayan, tekil gebelikler çalışmaya dahil edilmiştir. 10-14, 20-24 ve son olarak 30-34 gestasyonel haftalarda kan örnekleri hastalardan toplanmış ve vitamin B12, folat ve homosistein seviyeleri hesaplanmıştır.
Sonuç: Vitamin B12 eksikliği (B12<126.5 pg/mL), birinci trimesterde %2.44, ikinci trimesterde %2.41 ve üçüncü trimesterde %2.27 gebede tespit edilmiştir. Folat ve homosistein seviyelerinde ikinci trimesterde ilk trimestere göre istatistiki olarak anlamlı düşüş saptanmıştır (p<0.001).
Tartışma: Homosistein seviyesi üçüncü trimesterde ilk trimesterle benzer olmasına rağmen, ikinci trimester göre hafif bir artış göstermiştir. Sonuç olarak, vitamin desteği bulunmamasına rağmen Türkiye'de yeni sağlık sistemi politikası ile Vitamin B12 ve folat eksikliği diğer ülkeler ile kıyasla belirgin az olarak bulunmuştur.

Anahtar kelimeler: *Vitamin B12; Folat; Homosistein; Eksiklik oranı*

ABSTRACT

Objective: Vitamin B12 (cobalamin) and folate are essential micronutrient for cellular growth and differentiation. In current sequential study, our aim is to determine the mean B12 and folate levels and prevalence of B12 and folate deficiency at each trimester in Turkish healthy pregnant women who did not use any supplementation.
Method: A prospective cross-sectional study was conducted between January-2016 and May-2017. Singleton pregnancies in the first trimester, not using any vitamin supplementation were included. Blood samples were collected at 10-14, 20-24 and at last 30-34 gestational weeks and vitamin B12, folate and homocystein levels were calculated.
Results: In first trimester, we found out that 2.44%, in second trimester 2.41% and in third trimester 2.27% of patients had vitamin B12 deficiency (B12<126.5 pg/mL). There was statistically significant decrease in folate and homocystein levels in the second trimester compare to first trimester (p<0.001).
Conclusion: The homocysteine level was found similar in the third compared to first trimester with a gradual increase from the second trimester value, this could be explained with the decrease in the B12 level in the third trimester. By the way, deficiency rates for B12 and folate were rare in Turkey after new health policy compared to other countries even without supplementation.

Keywords: *Vitamin B12; Folate; Homocystein; Deficiency rate*

INTRODUCTION

Vitamin B12 (cobalamin) is essential micronutrient for cellular growth and differentiation. With folic acid, B12 is fundamental for DNA, RNA, protein and lipid synthesis known as one carbon metabolism (1, 2). In this manner, vitamin B12 and folate are required cofactors for converting homocystein to methionine thus neurotransmitter and phospholipid synthesis.

The main causes for Vitamin B12 deficiency are malabsorption and malnutrition (3) and this leads to anemia, cognitive dysfunction and dementia (4, 5). In addition to that, in pregnant women B12 is essential for embryogenesis and placental development. Both low B12 and folate concentrations are related to neural tube defects (6), spontaneous abortion (6), preeclampsia (7, 8) and preterm birth (9) because of elevated homocystein levels. In recent studies (10, 11), because B12 is assigned in lipid metabolism, the deficiency of B12 resulted in maternal adiposity, maternal and offspring insulin resistance. Although some studies in literature showed the relationship between B12 deficiency and low birth weight (12), this is equivocal (13, 14).

The prevalence of vitamin B12 deficiency is 1.4% all in anemic individuals and 18% in macrocitic anemia (15). With increasing age, the deficiency prevalence also increases. Apart from that, in pregnant women, the B12 deficiency prevalence is much more higher than the normal population. In a recent systematic review, B12 deficiency among pregnant women across the world was 20-30% (16).

In the current sequential study, our aim is to determine the mean B12 and folate levels in healthy pregnant population and prevalence of B12 and folate deficiency at first, second and third trimester in Turkish healthy pregnant women who did not use any supplementation.

MATERIAL AND METHODS

Study Population

This prospective cross-sectional study was conducted in Gazi University Faculty of Medicine, Department of Obstetrics and Gynecology, between January-2016 and May-2017. All healthy pregnant women were questioned for the following inclusion criterion: (1) female age 18 – 45, (2) singleton pregnancy in the first trimester, (3) not using any vitamin supplementation. Multiple pregnancies, pregnancies with obstetric complications such as hypertensive disorder and gestational diabetes and with maternal diseases (especially diseases cause malabsorption like Crohn disease) and tobacco smoking were excluded from the study. All pregnant women used folic acid supplementation in first trimester. With pregnancies who had B12 deficiency (B12<126.5 pg/mL) at the first, second or the third trimester, the trimester specific deficiency prevalence was calculated and after, those patients were also excluded from the study during follow up. All the patients in the study had same dietary information to maximize B12 and folate intake and absorption. Approval from institutional review board from Gazi University Faculty of Medicine was obtained (Approval No: E-15-558). All participants gave informed consent before the study began.

Blood Sampling

Blood samples were collected in standard fashion at first 10-14 gestational weeks, secondly in 20-24 gestational weeks and at last 30-34 gestational weeks. Blood vitamin B12, folate and homocystein were analyzed. Vitamin B12 was analyzed with Beckman Coulter test, DXI800. B12 deficiency was accepted as <126.5 g/d. Blood was collected and analyzed with method of direct chemiluminescence (ADVIA Centaur equipment, Siemens). All participants in this study had non-fasting blood samples.

Statistical Analysis

Statistical analyses were performed by SPSS v21.0 (IBM SPSS Inc., Chicago, IL) and $p < 0.05$ was defined as significant. Continuous variables were presented as mean followed by standard deviation and median with maximum–minimum. The normality of the variables was tested with Kolmogorov–Smirnov test. For statistical analysis Kruskal-Wallis and Wilcoxon Signed Rank Test were used. To determine the deficiency prevalence of B12, we defined the patients with B12 <126.5pg/mL in each trimester. The 2,5th, 5 th , 10 th , 90 th , 95 th and 97,5th percentile values of B12 and folate were also defined for each trimester.

RESULTS

Of the 221 pregnant women were requested for the study, 164 were appropriate for the final evaluation according to inclusion and exclusion criterion. The mean female age was 32.6±7.6 years. Due to severe deficiency and missed follow up, in total of 40 patients (4 had B12< 126.5 pg/mL and 37 missed follow up or had obstetric complications) in the 20-24 gestational week and 36 patients (36 missed follow up or had obstetric complications) in 30-34 gestational week were excluded.

In first trimester, we found out that 2.44% (4/164) of patients had B12<126.5 pg/mL (who were excluded from the study at the beginning). In second trimester 2.41% of patients (3/124) and in third trimester 2.27% of patients (2/88) had B12<126.5 pg/mL. The mean value of B12 in the first, second and third trimester was 325.43, 261.50 and 215.33 pg/mL, respectively. They were statistical significantly different (p<0.001). The mean values for folate and homocystein for each trimester were 26.0/ 15.70/ 12.40 ng/L and 8.44/ 5.98/ 7.42 umol/L, respectively. When the subgroup analysis was performed, median and maximum- minimum values were given in Table 1. There was statistically significant decrease in folate and homocystein levels in the second trimester compare to first trimester (p<0.001). In addition to that, the B12 and folate levels were decreased in the third trimester compare to first trimester (p<0.001, p=0.01, respectively), but homocystein stayed comparable (p=0.169). On the other hand, the difference in the B12 and folate levels between second and third trimesters were not statistically significant (p=0.105, p=0.5, respectively). There was an increase in the homocystein level in the third trimester compare to second trimester but it was not statistically significant (p=0.6).

The 2,5th ,5th , 10th , 90th , 95th and 97,5th percentile values of Vitamin B12 and folate for each trimester was shown in Table 2.

Table 1 Mean and median values for B12, folate and homocystein in first, second and third trimester and their p values.

	1st Trimester		**2nd Trimester**		**3rd Trimester**		**p**
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Vit B12 (pg/mL)	325,4± 138,4	287,2 (22.0-1080.0)	261,5± 86,9	242,0 (45.9-503.8)	215,3±72,8	196,7 (105.3-400.0)	0.00 0.00[a] 0.00[b] 0.105[c]
Folate (ng/mL)	26,0± 28,0	17,48 (5.4-224.0)	15,7± 10,6	12,24 (4.7-59.0)	12,4±0,8	11,90 (2.4-20.0)	0.00 0.01[a] 0.01[b] 0.5[c]
Homocystein (umol/L)	8,4± 3,8	8,0 (1.0-22.0)	5,9 ± 3,1	5,0 (2.0-18.0)	7,4±5,5	5,0 (3.0-23.0)	0.00 0.000[a] 0.169[b] 0.6[c]

a Comparison of first and second trimester
b Comparison of first and third trimester
c Comparison of second and third trimester

Table 2: Percentiles of vitamin B12 and Folate values in each trimester

	1st Trimester		**2nd Trimester**		**3rd Trimester**	
Percentiles	B12	Folate	B12	Folate	B12	Folate
2,5	142,5	6,5	90,8	5,3	107,2	2,4
5	176,2	7,0	150,0	6,0	118,0	4,2
10	202,0	8,7	168,0	7,3	130,2	6,3
50	286,0	17,5	241,1	12,1	196,2	11,7
90	473,4	49,3	384,4	20,0	310,1	19,6
95	555,8	57,1	430,0	44,0	336,8	19,8
97,5	604,0	85,4	456,4	48,7	393,0	19,9

DISCUSSION

Vitamin B12 and folate are essential micronutrients both for fetal development and maternal health in pregnancy. Although in neither guidelines screening of these micronutrients in pregnancy have not been recommended, deficiency of them could cause severe structural anomalies like neural tube defect. In this manner, levels of these vitamins were important and folic acid supplementation routinely suggested preconceptional and in first trimester strongly (17).

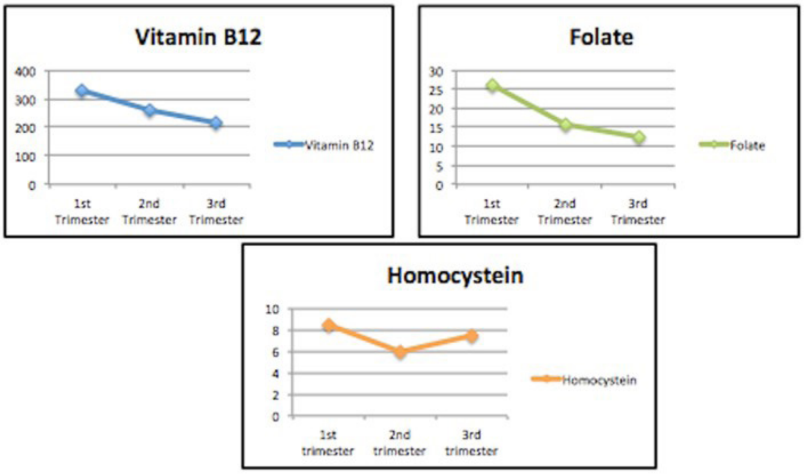
Our aim in this study was to find vitamin B12 deficiency prevalence for trimesters in Turkish healthy pregnant population. For this reason, only the healthy pregnant women without any vitamin supplementation were included in the study. In literature, B12 deficiency was higher in pregnant women than non-pregnant healthy population (%30 vs 1.4). This could be because of dilution (increase in plasma volume) in pregnancy or increase in usage. This deficiency rates are strongly related with nutrition so the rates were vary between countries. Some second trimester B12 deficiency rates in India (16), Venezuela(18) and South Korea(19) were 64%, 59% and 46%, respectively. In addition to that, in Colombia (20), Argentina (21) and United Kingdom (22) deficiency rates were 30%, 49% and 20%, respectively. In current study, our deficiency rates (2.41%) were lower than literature, this could be occurred because of all pregnant women in current study were healthy and had dietary advise at the beginning of the pregnancy for supplying adequate vitamin B12 and folate.

In addition to that, in other studies threshold values for B12 deficiency were determined as 221, 146 and 150 pg/dl. When we calculate 10th percentile values for each trimester in the current study, they were 202, 168 and 130 pg/dl for the first, second and third trimester respectively. However, in this study the threshold were accepted as 126.5 pg/dl because of technical kit. This could be one of the reason for our low prevalence in vitamin B12 deficiency.

In past years there were prevalence studies for B12 in Turkish pregnant women. According to Halicioglu et al in 2012 (23), the deficiency rate was 47.6% and according to Balcı et al in 2014 (24), the deficiency rate was 70.8%. The decline in deficiency rate in Turkey could because the change in health policy. Since past three years, all non-pregnant individuals have been screened for B12 deficiency and treated according to the results. By this way, the population deficiency rates were declined abruptly. Our first trimester deficiency rate was only 2.44% which showed adequate B12 intake prior to pregnancy.

The mean level of vitamin B12 was highest in the first trimester. B12 values decreased gradually between 10-14 / 20-24 and 30-34 gestational weeks (Figure 1). There was statistically significant decrease in B12 levels in the second trimester compare to first trimester. The main reason for the difference could be dilution in pregnancy.

Figure 1: Mean values of Vitamin B12, folate and homocystein values from first to third trimester



There is inverse relationship between homocysteine and Vitamin B12- folate values and the harmful hyperhomocysteinemia is mostly due to deficiency of vitamin B12 and folate (25). We found both homocysteine and B12 levels decreased in the second trimester compared to first trimester, this could be due to physiological hemodilution. The homocysteine level was found similar in the third trimester compared to first trimester with a gradual increase from the second trimester value, this could be explained with the decrease in the B12 level in the third trimester.

The strongest part of our study was the selected healthy population did not use any vitamin supplementation during pregnancy that could change the B12 and folate levels. Another valuable part of the study was, this was the first prospective study in Turkey that the same population was followed up till birth. The study population could represent the Turkish population thus our clinic is a reference hospital in Turkey and had several patients from different sides of the country.

In conclusion, this is a pioneer study to determine the deficiency rates for B12 and folate during pregnancy and its effects on homocystein without using any vitamin supplementation. Our study showed that the deficiency rates for B12 and folate were rare in Turkey compared to other countries even without supplementation. Although B12 and folate stayed in normal range, non-significant decreases caused non-significant increase in homocystein level. By this way, giving routine supplementation especially for B12 was redundant. Pregnancy outcomes according to B12 and folate levels should be investigated in order to reach to more informative conclusions.

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