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Habitüel Abortusta Vitamin B_{12} ve Folatın Rolü

The Role of Vitamin B_{12} and Folate in Habitual Abortion

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ÖZ

Amaç: Habitüel abortus etiyolojisinde birçok patofizyolojik mekanizma tanımlanmış olsa da vakaların yarısının nedeni hala aydınlatılamamıştır. Folat ve B_{12} vitaminin erken gebelik komplikasyonları üzerinde etkili olduğu bildirilmektedir. Biz de çalışmamızda habitüel abortus ile folat ve vitamin B_{12} düzeyleri arasındaki ilişkiyi ortaya koymayı amaçladık.

Materyal ve Metot: Habitüel abortus hikayesi olan 124 gebe ve böyle bir hikayesi olmayan 242 gebe olmak üzere, toplam 366 hasta çalışmaya dahil edilmiştir. Hastaların yaş, gestasyonel hafta, boy, kilo, vücut kitle indeksi (VKİ), gebelik, parite, düşük ve yaşayan çocuk sayısı ve vitamin B_{12} ve folat seviyeleri değerlendirildi.

Bulgular: Çalışma sonuçlarımıza göre çalışma grubunun yaş, gravida ve düşük sayıları kontrol grubundan anlamlı olarak yüksek bulunmuştur ($p<0,05$). Grupların boy, kilo ölçümleri, vitamin B_{12} ve folat ölçümleri arasında istatistiksel olarak anlamlı bir fark bulunmazken ($p>0,05$), çalışma grubunun VKİ ölçümleri ve yaşayan çocuk sayısı kontrol grubundan anlamlı olarak düşük bulunmuştur ($p<0,05$).

Sonuç: Çalışmamızın sonuçlarına göre vitamin B_{12} ve folat ile habitüel abrtus arasında herhangi bir anlamlı ilişki saptamadık. Konuyla ilgili literature katkıda bulunacak daha büyük örneklemli prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Folat, habitüel abort, vitamin B_{12}

ABSTRACT

Objective: Although several pathophysiological mechanisms are defined in the etiology of habitual abortion, still causes half of the cases haven't been clarified yet. It has been reported that folate and vitamin B_{12} are effective in early pregnancy complications. In our study, we aimed to reveal the relationship between habitual abortion with folate and vitamin B_{12} levels.

Materials and Methods: We included our study 124 pregnant having habitual abortion history and 242 pregnants without this, a total of 366 patients. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion, and living children count and vitamin B_{12} and folate levels of these pregnants were evaluated retrospectively.

Results: The ages, gravidity, and abortion counts of the study group were significantly higher than the control group ($p<0.05$). While the weight, height measurements, vitamin B_{12}, and folate measurements showed no significant difference ($p>0.05$) between groups, the BMI measurements, living children count, and of the study group were significantly lower than the control group ($p<0.05$).

Conclusion: According to our results, we didn't find any relationship between habitual abortion with folate and vitamin B_{12} levels. Further larger sample-sized and prospective studies are required to contribute to the literature about this issue.

Keywords: Folate, habitual abortion, vitamin B_{12}

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INTRODUCTION

Habitual abortion is defined as 3 or more consecutive pregnancy losses before the 20th gestational week.[1] There is no consensus in the number of abortions in the definition of habitual abortion. The United Kingdom Royal College of Obstetricians and Gynaecologists (RCOG) handles habitual abortion as three or more consecutive pregnancy losses. Because RCOG evaluates the definition of abortion as a loss before the 24th week of pregnancy and includes biochemical pregnancies in the definition of habitual abortion.[2] German, Austrian and Swiss Gynecology and Obstetrics associations also handle three or more pregnancy loss as habitual abortion,[2] while The American Society for Reproductive Medicine (ASRM) defines habitual abortion as two or more clinical pregnancy loss, that is, biochemical pregnancy loss is not included in the definition.[3] Habitual abortion is an important reproductive health problem affecting 2 to 5% of couples. Although etiologies include immune, genetics, endocrine, infectious and genetic factors, metabolic and anatomical abnormalities, half of the cases cannot be explained despite detailed examinations.[4] Hence, nowadays with the developing technology, it is thought to shed light on etiological factors that cannot be clarified by Deoxyribonucleic acid (DNA) methylation, proteomics, and micro Ribonucleic acid (RNA) assays.[5] It has been reported that folate and vitamin B_{12}, which are effective in cell division, growth, and differentiation, and fetoplacental development, are also responsible for early pregnancy complications, especially through DNA methylation.[6] In pregnancy, a tendency to folic acid deficiency is occurred due to increased metabolic demand due to the acceleration of cell proliferation or a decrease in dietary intake.[7] In developing countries, vitamin B_{12} levels decreased due to a vegetarian diet.[8] Hemodilution in pregnancy also contributes to this.[9] When folate and vitamin B_{12} levels are not brought to normal levels, pregnancy complications such as neural tube defects, spontaneous abortion, preeclampsia, and preterm delivery can be observed.[10,11]

In our study, we aimed to reveal the relationship between habitual abortion with folate and vitamin B_{12} levels.

MATERIALS AND METHODS

The ethical committee approval of Health Sciences University Samsun Training and Research Hospital Medical Specialization Training Board, was obtained for conducting the research (Date: 27/05/2020, decision no: GOKA 2020/7/30). This study was performed within the guidelines of the Helsinki Declaration.

366 patients who applied to Samsun Gynecology and Obstetrics Hospital and Health Sciences University Samsun Training and Research Hospital Gynecology and Obstetrics Department between January 2016 and January 2019 were included in the study. One hundred twenty-four patients, who had 5-19 weeks gestational age (median 10 weeks) pregnancy and whose heartbeat could not be detected in the intact gestational sac in transvaginal ultrasonography and with two consecutive 7-10 weeks of pregnancy loss were included in the habitual abortion group. Those with uterine abnormality, chromosome abnormality, thyroid dysfunction, toxoplasma, rubella, cytomegalovirus, and herpes virus infection, diabetes, hypertension, and autoimmune disease were excluded from the study. Two hundred forty-two women with a live, healthy pregnancy that gestational age-matched (6-18 weeks median 10 weeks) with the study group and having no miscarriage history were included in the control group. The study was planned as a retrospective cross-sectional study. Maternal and gestational age, height, weight, body mass index (BMI), gravidity, parity, abortion, and living children count, vitamin B_{12}, and folate levels of participants were obtained from previous hospital records and evaluated. Vitamin B_{12} and folic acid levels were determined using the electrochemiluminescence method in the immunity 2000 device with the determination kit by the DPC company.

***Statistical Analysis*:** The sample size was determined for α: 0.05 and ß: 0.80. NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used when evaluating the study data. The suitability of quantitative data for normal distribution was tested by Kolmogorov-Smirnov, Shapiro-Wilk test, and graphical evaluations. Student's t-test was used for comparing two groups of normally distributed quantitative data, and the Mann Whitney U test was used for two-group comparisons of non-normally distributed data. Significance was considered at least $p<0.05$.

RESULTS

The study was conducted with 366 female cases. The ages of the cases ranged between 17 and 44, with an average of 28.46±5.88 years. Weight measurements of the cases ranged from 41 to 110 kg, with an average of 69.09±12.48 kg; their height varied between 1.4 and 1.92 m, with an average of 11.59±0.09 m; BMI measurements ranged from 15.9 to 42.2 kg / m2, with an average of 27.41±4.65 kg / m2. Gravidity of the cases range from 1 to 10, and the median was 2; the parity numbers ranged from 0 to 6, the median was 1; the number of abortions ranged from 0 to 5, the median was 0 and the living children count ranged from 0 to 5, the median was 1 child.

As shown in Table 1, the ages ($p=0.002$; $p<0.01$), gravidity ($p=0.001$; $p<0.05$) and abortion counts ($p=0.001$; $p<0.01$) of the study group were found significantly to be higher than the control group. While the weight and height measurements of the cases showed no statistically significant difference ($p>0.05$) between groups; the BMI measurements ($p = 0.010$; $p<0.05$), living children count ($p= 0.001$; $p<0.01$) of the study group was significantly lower than the control group.

Moreover, Vitamin B_{12} and Folate measurements of the cases did not show a statistically significant difference by groups ($p>0.05$) is presented in Table 2.

Table 1. Evaluation of demographic features by groups.

Measurements	Total (n=366) Min-Max(median) Mean±SD	Habitual abortion (n=124) Min-Max(median) Mean±SD	Live pregnancy (n=242) Min-Max(median) Mean±SD	p
Age (years)	17-44 (28)	17-44 (31)	18-42 (27)	[a]0.002**
	28.46±5.88	30.32±6.65	27.73±5.39	
Weight (kg)	41-110 (68)	41-110 (67)	45-110 (69)	[a]0.76
	69.09±12.48	66.93±11.62	69.85±12.71	
Height (m)	1.4-1.92 (1.6)	1.45-1.75 (1.6)	1.4-1.92 (1.58)	[a]0.06
	1.59±0.09	1.60±0.05	1.59±0.10	
BMI (kg/m^2)	15.9-42.2 (27)	17.1-36.1 (25.4)	15.9-42.2 (27.1)	[a]0.001**
	27.41±4.65	26.24±4.49	27.80±4.65	
Gravidity	1-10 (2)	2-8 (3)	1-10 (2)	[b]0.001**
	2.47±1.66	2.63±1.44	2.41±1.73	
Parity	0-6 (1)	0-3 (1)	0-6 (1)	[b]0.074
	0.94±1.12	0.71±1.15	1.01±1.10	
Abortion	0-5 (0)	0-5 (2)	0-0 (0)	[b]0.001**
	0.42±0.75	0.61±0.88	0	
Living children count	0-5 (1)	0-2 (1)	0-5 (1)	[b]0.001**
	0.98±1.08	1.03±1.16	0.97±1.06	

[a]:Student t Test; [b]:Mann Whitney U Test; **: $p<0.01$; BMI: Body Mass Index; Min: Minimum; Max: Maximum; SD: Standard deviation.

Table 2. Evaluation of laboratory findings by groups.

Measurements	Total (n=366) Min-Max(median) Mean±SD	Habitual abortion (n=124) Min-Max(median) Mean±SD	Live pregnancy (n=242) Min-Max(median) Mean±SD	p
Vitamin B_{12} (pmol/L)	80.1-1078 (275.5)	101-1078 (279.5)	80.1-715 (270)	[b]0.850
	306.87±124.25	295.12±91.83	311.36±134.49	
Folate (µg)	3.1-26.7 (17.9)	3.1-25.9 (15.8)	3.3-26.7 (17.5)	[a]0.056
	17.31±5.99	16.88±6.39	17.47±5.83	

[a]: Student t Test; [b]: Mann Whitney U Test; Min: Minimum; Max: Maximum; SD: Standard deviation.

DISCUSSION AND CONCLUSION

Habitual abortion is an entity that adversely affects couples both socially and psychologically, but 50% of its etiology has not yet been elucidated.[4] The role of folic acid and vitamin B_{12} is on the agenda to clarify the unexplained part of the etiology.[12,13] Folate and vitamin B_{12} play a key role in cell proliferation maturation due to their critical role in DNA synthesis.[14] These vitamins are known to play an important role in single carbon metabolism reactions where S-adenosine methionine becomes a methyl donor.[15] Vitamin B_{12} plays a role in the synthesis of methionine synthase and methylmalonyl coenzyme-succinyl Ko A, which provides a synthesis of methionine from homocysteine.[16]

Its deficiency causes secondary folic acid deficiency and homocysteinemia and methylmalonic acidemia (MMA). Also, chromatin structure and gene expression change as a result of a decrease in DNA methylation.[17] Folate is also involved in transmethylation and transsulfuration reactions that play a role in the metabolism of many amino acids and its deficiency increase homocysteine levels.[18] Folate and vitamin B_{12} deficiency and homocysteinemia have been shown to give rise to preeclampsia, intrauterine growth retardation, preterm labor, habitual abortion, gestational diabetes, and rise birth defects such as neural tube defect.[19,20]

In our study, vitamin B_{12} and folic acid measurements did not show a significant difference between the group with habitual abortion and the control group. In a study conducted by Sütterlin et al.[21,29] patients with at least 3 or more abortions, including 29 biochemical pregnancies and 29 healthy control groups with similar characteristics were prospectively compared and no statistically significant difference was found between folate and vitamin B_{12} levels between groups. However, it was shown that folate levels decreased significantly as the number of abortions increased. In fact, since patients with 3 abortions and above were included in the case group, although there was a significant difference expectation, the small sample size may have affected the result. In our study, results were obtained parallelly with this study.

In a prospective study by Hübner et al.,[19] they included in the study 43 patients with 2 or more pregnancy losses and 32 women with healthy pregnancies in the control group. While vitamin B_{12} levels were found to be low in the group with habitual abortion, methylmalonic acid (MMA) levels were found to be significantly higher in the case group and no significant difference was found between folate and homocysteine levels. In this study, it is noteworthy that vitamin B_{12} levels were lower and MMA levels were higher in patients who had never given birth. In the study we conducted, no significant difference was found between groups regarding folate levels similarly to the result of this and our results regarding vitamin B_{12} values do not show parallelism.

In a case-control study including 107 patients with three or more pregnancy losses and 343 women who had 2 healthy births were appointed as the control group by Puri et al.,[12] folic acid, vitamin B_{12}, homocysteine levels and Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism was studied. Vitamin B_{12} levels of the habitual abortion group were found to be significantly lower, while homocysteine levels were significantly higher. While a negative correlation was found between homocysteine and vitamin B_{12}, it was obtained that hyperhomocysteinemia increased the risk of habitual abortion by 7.02 times, and vitamin B_{12} deficiency increased by 16.39 times. The mutant T allele was found not to increase the risk of habitual abortion. In this study, it was emphasized that vitamin B_{12} deficiency poses a serious risk for habitual abortion, and although the results of our study do not show agreement with vitamin B_{12}, there is a correlation with our study in terms of folate levels.

In a case-control study including 50 patients with at least 2 abortions and 50 healthy women without any adverse pregnancy, outcome history was determined as the control group by Al-Bedri et al.,[13] folate, vitamin B_{12}, and homocysteine levels were evaluated. In the case group, folate and vitamin B_{12} levels were found to be significantly lower while homocysteine levels were found to be higher. In this study, it was emphasized that vitamin B_{12} is the most specific and sensitive marker among the parameters tried to predict habitual abortion. However, the results of this study do not correlate with our results.

In a case-control study conducted by Abd-Ellatef et al.,[22] 60 patients with at least 2 pregnancy losses and 20 women without a history of abortion were included the study. Vitamin B_{12} levels of the habitual abortion group were found to be significantly lower and homocysteine levels of them were significantly higher. Besides, a negative correlation was noted between these two parameters.

When we look at the studies in the literature, different results were obtained depending on the differences in sample size and habitual abortion definition

criteria. However, it has been emphasized that vitamin B_{12} deficiency and hyperhomocys- teinemia increase the risk of habitual abortion. The reason why the studies in the literature have contradictory results regarding the correlation between folate levels and habitual abortion might be due to the lack of efficiency of folate on metabolic pathways despite normal plasma levels because of enzyme mutations (the most common type of them is MTHFR polymorphism) in folate metabolism.[18] Could not be assessing homocysteine levels of all participants due to the retrospective feature of the study leads to limitation. Also, because of the unavailability of the data of participants' folate replacement, we could not exclude the individuals having folate supplements from the study. This situation causes another limitation. However, compared to similar studies in the literature, the fact that the sample size was larger than most of them makes the study strong.

When the cause of the habitual abortion cannot be determined, it causes the family to despair and decrease motivation for the will for pregnancy again and cause difficulties in applying the treatment.

According to our study results, there was no significant relationship between habitual abortion with vitamin B_{12} and folate. However, larger sample-sized and prospective studies are required to contribute to the literature about this issue. Although the folic acid supplement is recommended for every woman planning a pregnancy, there is no such recommendation for vitamin B_{12}. However, it is vital to give a replacement for any woman with a pre-pregnancy deficiency.

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