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# EVALUATION OF RELATIONSHIP BETWEEN PAIN SCORE AND SERUM VITAMIN B12 AND FOLATE LEVELS IN FIBROMYALGIA PATIENTS

# FİBROMİYALJİ HASTALARINDA VİTAMİN B12 VE FOLAT DÜZEYLERİ İLE AĞRI SKORLARI ARASINDAKİ İLİŞKİNİN İNCELENMESİ

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## Öz

Amaç: Fibromiyalji sendromu (FMS), yaygın kas iskelet sistemi ağrısı ile sabah sertliği, yorgunluk, uyku bozukluğu, düşük ağrı eşiği, bilişsel işlev bozukluğu, anksiyete ve depresif ataklar gibi ilave semptomlar ile karakterize, yaygın bir romatolojik bozukluktur. Folat ve B12 vitamini, tek karbon metabolizması ve ilişkili hastalıklarla ilgili önemli işlevleri paylaşan metabolik ve klinik olarak birbirleri ile ilişkili vitaminlerdir. Yapılan çalışmalarda, folat ve B12 vitamininin ağrı ve ağrıya bağlı rahatsızlıklarla ilişkili olduğu gösterilmiştir. Bu çalışmada, FMS hasta grubu ile kontrol grubu arasındaki serum vitamin B12 ve folat düzeylerini karşılaştırmak ve FMS hastalık patogenezine yeni kanıtlar sunmayı amaçlamaktayız.

Gereç ve Yöntem: Çalışmaya 46 kadın FMS hastası ve 46 sağlıklı birey alındı. Hastaların dosyalarından B12 vitamini, folat, eritrosit sedimantasyon hızı (ESR) ve C-reaktif protein (CRP) ve görsel analog skalası (VAS) skorları dahil olmak üzere klinik bilgi ve biyokimyasal belirteçler elde edildi.

Bulgular: İki grup arasında vitamin B12, folat ve ESR düzeylerinde anlamlı farklılık olmadığı bulundu (p> 0.05). Serum CRP düzeyleri, FMS grubunda anlamlı olarak arttığı gözlendi (p = 0.027). Hasta grubunda, VAS skorları ile biyokimyasal belirteçler arasında korelasyon olmadığı gözlendi.

Sonuç: Bu çalışma, B12 vitamini ve folat düzeylerinin FMS patogenezinde yer almadığını göstermiştir. FMS hastalarında ağrı algılaması ile tek karbon metabolizma belirteçleri arasında korelasyon gözlenmemiştir.

**Anahtar Kelimeler**

Fibromiyalji, Vitamin B12, Folat, Tek karbon metabolizması, Ağrı

## Abstract

Aim: Fibromyalgia syndrome (FMS) is a common rheumatologic disorder which is characterized by chronic widespread musculoskeletal pain and several additional symptoms including morning stiffness, fatigue, sleep disorder, low pain threshold, cognitive dysfunction, anxiety and depressive episodes. Folate and vitamin B12 are two metabolically and clinically related vitamins that share some important functions related to the one-carbon metabolism and related diseases. Studies have revealed that vitamin B12 and folate are associated with pain and pain-related disorders. The aim of this study was to compare serum vitamin B12 and folate levels between FMS patient group and the control group and to present a new evidence of pathogenesis FMS disease.

Material and Methods: 46 female FMS outpatients and 46 healthy individuals were enrolled in the study. Clinical information and biochemical markers including vitamin B12, folate, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and visual analogue scale (VAS) scores were obtained from the patients' medical files.

Results: There were no significant differences in vitamin B12, folate and ESR levels between the two groups (p >0.05). Serum CRP levels was significantly increased in FMS group (p =0.027). There was no correlation between VAS scores and biochemical markers in patients group.

Conclusions: The results of this study have indicated that vitamin B12 and folate levels are not involved in the pathogenesis of FMS. No relevant correlations between the perception of pain and one carbon metabolism biomarkers were observed in FMS patients.

**Keywords:**

Fibromyalgia, Vitamin B12, Folate, One carbon metabolism, Pain

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## Introduction

Fibromyalgia syndrome (FMS) is a common rheumatologic disorder which is characterized by chronic widespread musculoskeletal pain and several additional symptoms including morning stiffness, fatigue, sleep disorder, low pain threshold, cognitive dysfunction, anxiety and depressive episodes (1). The global mean prevalence of FMS in the general population varies between 2% and 8% with a female-to-male ratio of 3 : 1, and the diagnosis is most often made in the middle and upper-middle age (2,3). The etiology and pathogenesis of FMS is still uncertain, but it is considered an interaction between neuroendocrine, metabolic and immunological factors in the development and progression of FMS disease. Vitamin and mineral deficiencies have recognized as risk factors (1).

Vitamin B12 is a multi-faceted vitamin and is involved in several metabolisms such as energy homeostasis, methylation reactions, fatty acid synthesis, DNA synthesis and regulation (4). Folate is a water-soluble vitamin that functions as co-factor in a variety of enzymatic reactions within the cell and is essential in many biochemical processes including amino acid metabolism, purine and thymidylate synthesis, and DNA methylation (5). Folate and vitamin B12 are two metabolically and clinically related vitamins that share some important functions related to the one-carbon metabolism and related diseases. Studies have revealed that vitamin B12 and folate are associated with pain and pain-related disorders (6, 7). The results of vitamin B12 and folate on fibromyalgia patients vary and are inconsistent. However, there have been no studies done on relationship between one carbon metabolites levels and pain scores in fibromyalgia patients.

The aim of this study was to compare serum vitamin B12 and folate levels between fibromyalgia patient group and the control group and to present a new evidence of pathogenesis fibromyalgia disease.

## Material and Methods

This pilot, retroprospective, case-control study was carried out from January 2017 to December 2017 at Department of Physical Medicine and Rehabilitation, Karabuk Training and Research Hospital, Karabuk, Turkey. 46 female outpatient fibromyalgia patients were enrolled in the study. The American College of Rheumatology criteria for FMS were used to establish the diagnosis of FMS (8). 46 female outpatient healthy volunteers, with normal radiological examinations and clinical histories and examinations, who visited the hospital for

routine physical examinations were included as controls. Controls were matched in terms of age and body mass index (BMI) with the FMS group. BMI values of the patient group and control group were in the normal range. Clinical information including age, course of disease and biochemical markers was obtained from the patients' medical files. The severity of the pain was self-assessed by using a linear visual analogue scale (VAS). The VAS scores reported for all patients were 0 (no pain whatsoever) and 10 (worst possible pain). The study was conducted in accordance with the ethical standards stated in the Declaration of Helsinki and was approved by the Research Ethics Committee of Karabuk University Medical School. Written and verbal informed consent was obtained from individuals prior to their participation in this study.

Participants with a history of smoking, acute or chronic infectious diseases, vitamin B12 and folate use, autoimmune, endocrine, or metabolic disorders, cardiovascular or cerebrovascular disease, demyelinating neurological diseases, peripheral neuropathies, diabetes mellitus, obesity (BMI >30), abnormal liver or renal function tests, malignancies, pregnancy, thrombosis, musculoskeletal surgery or injury within the previous two months, other musculoskeletal disease (rheumatoid arthritis etc.) were excluded from the study.

Blood samples were drawn from the antecubital vein of all participants after overnight fasting state. After clotting, blood samples were centrifuged at 2000×g for 10 min and serum was separated. All specimens were collected and analyzed within less than one hour after the sampling. Serum vitamin B12 and folate levels were determined by a Siemens ADVIA Centaur XP automated chemiluminescence immunoassay analyzer in the central laboratory of our institution which is routinely checked every day. According to the instruction of Siemens Laboratories, the reference range for serum folate was 3–18 ng/mL. Serum folate levels <3 ng/mL have traditionally been considered a sign of inadequate folate (9). The normal reference values for vitamin B12 were 211-971 pg/mL. ESR was determined by the Westergren method. ESR levels were considered to be elevated if they were greater than 20 mm/h. CRP level was determined by the immunoturbidimetric method. CRP levels were considered to be elevated if they were greater than 5 mg/dl.

**Statistical Analysis**

All statistical analyses were performed using SPSS 22.0 software (Chicago, IL, USA). All data were tested for normal distribution using the Shapiro Wilk test. Data are expressed as mean±standard deviation. Student's t-test for independent samples was used to analyze and to

compare the groups. The correlations between the variable pairs were analyzed using Pearson's correlation test. Differences between groups were significant when p< 0.05.

**Table 1:** Serum levels of biochemical markers and age in fibromyalgia patients and the controls

| | **Fibromyalgia patients (*n*=46)** | **Controls Group (*n*=46)** | ***p* value** |
|---|---|---|---|
| **Age (years)** | 48.8 ± 10.1 | 47.9 ± 14.4 | 0.726 |
| **Folate (ng/mL)** | 6,61 ± 2.04 | 6.96 ± 2.07 | 0.418 |
| **Vitamin B12 (pg/mL)** | 311.24 ± 91.19 | 364 ± 200.58 | 0.108 |
| **CRP (mg/dL)** | 3.19 ± 3.35 | 1.96 ± 1.55 | **0.027*** |
| **ESR (mm/h)** | 20.83 ± 10.55 | 17.96 ± 7.75 | 0.141 |

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, *: means statistically significant

**Table 2:** Correlations between biochemical markers and VAS score in fibromyalgia patients

| | | **CRP** | **ESR** | **Folate** | **Vitamin B12** | **VAS Score** |
|---|---|---|---|---|---|---|
| **CRP** | *r* value | 1 | **,569(**)** | ,062 | ,027 | -,080 |
| | *p* value | | ,000 | ,685 | ,857 | ,598 |
| **ESR** | *r* value | **,569(**)** | 1 | -,011 | -,172 | -,161 |
| | *p* value | ,000 | | ,943 | ,252 | ,285 |
| **Folate** | *r* value | ,062 | -,011 | 1 | ,277 | ,122 |
| | *p* value | ,685 | ,943 | | ,063 | ,419 |
| **Vitamin B12** | *r* value | ,027 | -,172 | ,277 | 1 | ,108 |
| | *p* value | ,857 | ,252 | ,063 | | ,474 |
| **VAS Score** | *r* value | -,080 | -,161 | ,122 | ,108 | 1 |
| | *p* value | ,598 | ,285 | ,419 | ,474 | |

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, *: means statistically significant

## Results

A total of 92 female outpatient patients (46 fibromyalgia and 46 healthy individuals) were included in the study. There was no significant difference between patient group with fibromyalgia and healthy controls in terms of age ($p$ = 0.726) (Table 1).

Mean folate levels were 6,61 ± 2.04 ng/mL in patients with fibromyalgia, vs. 6.96 ± 2.07 ng/mL in the control group ($p$ = 0.418). Vitamin B12 levels were 311.24 ± 91.19 pg/mL in patients group and 364 ± 200.58 in the control group ($p$ = 0.108). Serum CRP levels were 3.19 ± 3.35 mg/dL in patients with fibromyalgia, vs. 1.96 ± 1.55 in controls ($p$ = 0.027). Erythrocyte sedimentation rate levels were 20.83 ± 10.55 mm/h in patients group and 17.96 ± 7.75 in the control group ($p$ = 0.141) (Table 1).

In patients with fibromyalgia had a positive correlation between serum CRP levels and erythrocyte sedimentation rate levels (Table 2). There was no correlation between VAS scores and biochemical markers in patients group (Table 2). There was also a positive correlation between serum CRP levels and erythrocyte sedimentation rate levels in controls group (Table 3).

**Table 3:** Relationship between vitamin B12 and folate and inflammatory markers in controls group

| | | CRP | ESR | Folate | Vitamin B12 |
|---|---|---|---|---|---|
| **CRP** | *r* value | 1 | **,499(**)** | -,142 | -,101 |
| | *p* value | | ,000 | ,345 | ,503 |
| **ESR** | *r* value | **,499(**)** | 1 | ,186 | ,139 |
| | *p* value | ,000 | | ,216 | ,356 |
| **Folate** | *r* value | -,142 | ,186 | 1 | -,075 |
| | *p* value | ,345 | ,216 | | ,618 |
| **Vitamin B12** | *r* value | -,101 | ,139 | -,075 | 1 |
| | *p* value | ,503 | ,356 | ,618 | |

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, *: means statistically significant

## Discussion

Fibromyalgia syndrome is a common rheumatic condition that causes in many patients widespread pain and joint tenderness and is frequently accompanied by additional somatic and cognitive/affective symptoms. FMS have many risk factors including age, gender, genetic predisposition, environmental factors, occupation, obesity, low physical activity, smoking, reduced biogenic amines, vitamin and mineral deficiency (10). However, the exact etiology and pathogenesis of FMS is still unclear. To our knowledge, this is the first study to show the relationship between one carbon metabolism biomarkers, serum folate and vitamin B12 levels and pain score in fibromyalgia patients. We demonstrated that serum vitamin B12 and folate levels did not significantly differ in sera of fibromyalgia patients and no correlation between pain score and biochemical markers.

One carbon metabolism involves both folate and methionine cycles and allows cells to produce one carbon unit (also known as methyl groups) and supports multiple physiological processes including purines and thymidine biosynthesis, amino acid homeostasis (serine, glycine, methionine), methylation reactions, homocysteine re-methylation, redox homeostasis and epigenetic maintenance (11). Disruptions in that metabolism contribute to the pathogenesis of many diseases, such as cancers, psychosis, Alzheimer's disease, and autism

(11, 12). Folate is a major dietary source of one carbon units whereas vitamin B12 is essential cofactor for enzymes involved in one-carbon metabolism (12). There are few studies in the literature that examine vitamin B12 and folate levels in FMS patients. Bengtsson et al showed normal cobalamin and folate levels in patients with primary fibromyalgia (13). Ortancil et al revealed similar serum vitamin B12 and folate levels in comparison with control group (14). Regland et al demonstrated in twelve outpatient fibromyalgia patients that cerebrospinal fluid (CSF) homocysteine levels were found to increase and vitamin B12 levels did not change in serum and CSF; however they found significant correlations between CSF vitamin B12 and fatigability and 15 items of Comprehensive Psychopathological Rating Scale (15). They also suggested that low CSF vitamin B12 levels could be important for re-methylation of homocysteine. Carvalho et al studied serum vitamin B12 levels in 29 fibromyalgia patients (16). They observed that vitamin B12 levels were within normal ranges and none of them was deficient for vitamin B12. These findings were consistent with our study. In our study, we showed that serum vitamin B12 and folate levels did not alter in female FMS patients. Our results suggest that vitamin B12 and folate are not related to the pathogenesis of FMS. Our results indicate that one carbon metabolism could not change through vitamin B12 and folate in FMS and suggesting that pathogenesis of disease is possibly through other different mechanisms.

In previous studies, the relationships between pain and vitamin B12 and folate levels have been demonstrated in many diseases and the use of these vitamins for treatment of painful diseases has shown a reduction of the pain scores (6,7,17). Methylcobalamine, a vitamin B12 analog, was found to have analgesic effects on diseases including nonspecific low back pain, neck pain, neuropathic pain, neuralgia, aphthous stomatitis and surgical procedures (7,17). In some countries, vitamin B12 is accepted as analgesic drugs (7). However, its mechanisms underlying the analgesic effect were poorly understood. Folic acid supplementation may have many potential benefits in preventing stroke and miscarriage and birth defects (spina bifida), and treatment of depression (18,19). Folic acid can also lead clinical improvement in pain and related symptoms (6). This theoretical background led us to hypothesize that vitamin B12 and folate could be used for reduction of pain in fibromyalgia patients. To the best our knowledge, relationship between pain scores and vitamin B12 and folate levels have not been investigated in patients with fibromyalgia. In our study, we did not find any correlations between pain scores and vitamin B12 and folate levels in FMS patients.

Our results suggested that the use of vitamin B12 and folate may not be effective in reducing pain in fibromyalgia patients.

CRP is a pro-inflammatory biomarker and reflects chronic systemic inflammation. It is involved as part of the diagnostic laboratory workup for many inflammatory rheumatic conditions such as rheumatoid arthritis, ankylosing spondylitis (20). Many studies have suggested a possible link between FMS and CRP levels (21-23). Xiao et al showed that serum CRP levels increased in FMS patients and correlated with BMI, ESR, IL-6 and IL-8 levels (21). Lund et al revealed increased CRP levels in male FMS patients (22). Rus et al demonstrated increased CRP levels in normal weight and over-weight FMS patients compared to controls (23). These findings were consistent with our study. In our study, we showed increased serum CRP levels in female FMS patients in comparison with control group. We also found similar ESR levels in FMS and controls. Our results support the hypothesis that inflammation may contribute to the pathogenesis of FMS. We also observed no relationship between pain scores and inflammatory markers including CRP and ESR.

In our study, patient and control groups consisted of same age, same gender and body mass index. They also had similar ethnicity, lifestyle and eating patterns. These factors may cause interferences to analyze vitamin B12 and folate levels and to evaluate fibromyalgia patients. In our study, BMI levels in patients group was normal range and there were no differences in BMI levels between two groups. In previous studies showed that serum vitamin B12 and folate levels changed in obesity patients (24). Obesity also contributes to fibromyalgia pathogenesis (25). Therefore, we included to study with normal BMI levels patients.

We must acknowledge some limitations of this study. The main limitation of our study is that it is cross-sectional nature and therefore its inability to establish causality. Also, the sample size of our study was small. Statistical tests usually require a larger sample size to confirm that the effect did not occur by chance alone. Further experimental and clinical studies with larger sample size are needed to identify the precise molecular mechanism underlying the interplay between these vitamins. Moreover, in our study, patient and control groups consist of female patients. Thus, the subjects may not be fully representative of the general fibromyalgia population, but instead representative of female outpatient fibromyalgia. In future studies, the levels of vitamin B12 and folate should be analyzed in general population. Despite these limitations, these data do form a basis for future studies examining

the relationship between fibromyalgia patients and serum vitamin B12 and folate levels and pain scores.

This pilot study demonstrates that serum vitamin B12 and folate levels did not differ in the FMS patients group and this indicates that vitamin B12 and folate are not involved in fibromyalgia pathogenesis. The study also suggested no association between perception of pain and one carbon metabolism markers in FMS. Further studies using larger populations and more detailed investigation will be needed to confirm our observations and to validate the current findings and to shed more light on the pathological functions that connect one carbon metabolism to fibromyalgia.

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