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Oxidative Stress Index and Vitamin C in The Diagnosis of Fibromyalgia Syndrome

Fibromiyalji Sendromu Tanısında Oksidatif Stres İndeksi ve C Vitamini

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Abstract

Aim: We aimed to evaluate whether serum Vitamin C levels and oxidative stress index (OSI) can be used in the diagnosis of Fibromyalgia Syndrome (FMS). Still there is no any specific laboratory marker for diagnosis of FMS and it mainly depends on clinical examination.

Material and Method: 53 female patients and 35 healthy female controls were enrolled to our cross-sectional study. VAS, BDI and FIQ were applied to the patients. Vitamin C levels were measured by HPLC. Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS) levels were determined by Spectrophotometric Assay method.

Results: While vitamin C and TAC levels of FMS patients were significantly lower than those of the controls, OSI was significantly higher in patients (p= 0.004, p= 0.009 and p= 0.048, respectively). There was a moderate positive and significant relationship between the tender points and FIQ, (r=0.505; p <0.001). The diagnostic performance of Vitamin C and TAC with ROC analysis were found: AUC=0.678, p= 0.003, AUC=0.639, p= 0.028 respectively.

Conclusion: Serum TAC and vitamin C levels decrease and OSI increases in FMS patients. It can be suggested that these three parameters can be considered as an additional tool for diagnosis of FMS.

Keywords: Fibromyalgia Syndrome, TAC, OSI, Vitamin C, Chronical pain

Öz

Amaç: FMS tanısı için spesifik bir laboratuvar belirteci yoktur ve esas olarak klinik muayeneye bağlıdır. Serum C vitamini düzeylerinin ve oksidatif stres indeksinin (OSI) Fibromiyalji Sendromu (FMS) tanısında kullanılıp kullanılamayacağını değerlendirmeyi amaçladık.

Gereç ve Yöntem: Kesitsel çalışmamıza 53 kadın hasta ve 35 sağlıklı kadın kontrol dahil edildi. Hastalara VAS, BDI ve FIQ uygulandı. C vitamini seviyeleri HPLC ile ölçüldü. Toplam Antioksidan Kapasitesi (TAC) ve Toplam Oksidan Durumu (TOS) seviyeleri Spektrofotometrik yöntemle belirlendi.

Bulgular: FMS hastalarının vitamin C ve TAC düzeyleri kontrollere göre anlamlı olarak düşük iken, hastalarda OSİ anlamlı olarak daha yüksekti (sırasıyla p= 0,004, p= 0,009 ve p= 0,048). Hassas noktalar ile FIQ arasında orta düzeyde pozitif ve anlamlı bir ilişki vardı (r=0,505; p <0,001). ROC analizi ile Vitamin C ve TAC'nin tanısal performansı sırasıyla AUC=0,678, p= 0,003 ve AUC=0,639, p= 0,028 olarak bulundu.

Sonuç: FMS hastalarında serum TAC ve vitamin C seviyeleri düşmekte ve OSİ artmaktadır. Bu üç parametrenin FMS tanısı için ek bir araç olarak düşünülebileceği önerilebilir.

Anahtar Kelimeler: Fibromiyalji Sendromu, TAC, OSİ, C Vitamini, Kronik Ağrı

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INTRODUCTION

Ethiopathogenesis and pathophysiology of Fibromyalgia Syndrome (FMS), still could not be defined clearly, but many mechanisms such as immunological, neurohormonal, psychological, environmental factors, genetic predisposition and oxidative stress are thought to play a role.[1,2] In FMS patients, reactive oxygen species (ROS) are produced by both plasma lipid peroxidation (LP) and protein carbonylation (PC) in high amounts.[3] Elevated oxidative stress and oxygenation abnormalities may cause some changes in muscle structure and metabolism of the patients. Abnormal oxygenation in the trigger point areas has been reported to be able to induce irregular processing of pain by the central nervous system. Since nervous and muscle tissues are particularly prone to damage with free radicals, oxidative stress and dysfunction of mitochondria have been blamed in FMS-related complaints by some authors.[4] It is thought that there are potent connections between oxidative stress and frequently seen symptoms of FMS such as; chronic widespread pain (CWP), sleep disturbances, restlessness, major depression, and fatigue.[5-6]

However, there are not enough studies researching whether daily dietary replacement of antioxidant vitamins, especially vitamin C, reduces the oxidative stress that occurs in FMS and improves the most commonly seen complaints. In addition, since there are no precise laboratory parameters, the diagnosis of this disease generally depends on clinical symptoms and some self-reported tools by patients. Although it is a known fact that oxidative stress generally increases in almost all inflammatory diseases, it is the hypothesis of this study that monitoring of serum vit C, TAC and OSI levels together in the course of FMS can be used as an additional tool in the diagnosis of the disease, and treatment of vitamin C deficiency may improve the prognosis of the disease.

The aim of this study is to investigate the diagnostic and prognostic roles of serum vitamin C, TAC and OSI levels in FMS patients.

MATERIAL AND METHOD

Patient Selection

This study is a cross-sectional study. Since FMS is known as females' disease we constituted our all participants of patient and control groups from females. Before the study we estimated our sample size of totally 88 participants, 53 of whom were in the patient group and 35 were in the control group, with 80% power, 5% margin of error and 0.55 effect size. We took approval of the local ethics committee of our University. In our study, , 53 female patients, whose vitamin C, TAC and TOS levels were not previously known, applied to the Physical Therapy and Rehabilitation Outpatient Clinic in 2018 and diagnosed according to the 2010 American Rheumatology College (ACR) diagnosis criteria were included in the study based only on their dignosis of FMS by the same physician to prevent bias. Likewise, the 35 healthy female volunteers from our hospital staff paired in terms of age and body mass index who were not previously known for their vitamin C, TAC and TOS levels, were randomly enrolled to the study as control group. Their vitamin C, TAC and TOS levels of both patient and control groups were not measured until all samples were collected. The demographic data, height and body weight measurements of the individuals were processed in the "Data Registration Form". After taking a detailed medical history, severity of pain of the FMS patients was self reported by patients with international and Turkish validations and reliabilities has been made tools; the Visual Analogue Scale (VAS), Beck Depression Inventory (BDI) [7] and Fibromyalgia Impact Questionnaire (FIQ) [8] without any intervention by researchers. TPNs of all patients were determined manually by a single physician by using finger pressure method.

BDI is a 21-item questionnaire evaluating the presence and severity of depression. The validity and reliability of BDI in Turkish population were assessed by Hisli et al.[9] The more severe depression is indicated with higher scores (0-9 points: minimum depression; 10-18 points: slight depression; 19-29 points: moderate depression; 30-63 points: severe depression).

FIQ is a 10-item evaluation tool measuring the status, prognosis, and outcomes of FMS patients. The validity and reliability of FIQ in Turkish population were assessed by Sarmer et al.[10] The total score ranges from 0 to 100, with a higher score indicating a greater effect of FMS on functionality.

Our exclusion criteria were pregnancy, breastfeeding, having chronic inflammatory, systemic or metabolic disease (such as diabetes mellitus, hypertension, cancer, ischemic heart disease), susceptibility to thrombotic or bleeding disorders, and body mass index ≥35 kg/m² as well as taking medications like anticoagulant, corticosteroid and vitamin supplements. Alcohol users and smokers were excluded. Informed consent form was obtained from those who accepted to participate in the study. The study was carried out in accordance with the 2008 Helsinki declaration.

Biochemical Analysis

After at least eight hours of fasting, morning bloods were taken from both groups for routine examination and centrifuged for 10 minutes at 3500 rpm after 30 minutes resting of samples. The serum was immediately separated and placed in small volume tubes and stored at -80°C until the working day. After the collection of samples of all participants was completed, all samples were studied by the same researchers at one session.

TAC and TOS Measurement

TAC and TOS measurements were made by spectrophotometric method using TAC Assay Kit (Rel Assay Diagnostics, Gaziantep, Turkey) and TOS Assay Kit (Rel Assay Diagnostics, Gaziantep, Turkey). Cobas c 501 module of the

Cobas 6000 (Roche, Basel, Switzerland) autonalizer was used in the measurement.

Calculation of OSI

Oxidative Stress Index (OSI) is the percentage ratio of TOS to TAC level. The degree of oxidative stress is indicated by OSI and is calculated by the following formula:

OSI (optional unit)=TOS (µmolH2O2 equivalent/l)/TAC (µmol Trolox equivalent/l.)

Serum Vitamin C levels measurement

Vit C serum levels were measured by High Performance Liquid Cromotography (HPLC). Chromatographic determinations were made at 253 nm length with Agilent 1100 HPLC-UV (Agilent Technologies, Palo Alto, California) equipped with 1100 series pump and UV-VIS detector. Injection volume was set to 30 µL. Internal standard and precipitation reagent were used. Stainless steel, Phenomenex, Luna C18 100A 150x4.6 mm 5um (Production location: California, USA) column was used at room temperature (20-25°C).

Statistical Analysis

G*Power 3.1.9 [11] was used to determine an adequate sample size, Based on the research of Claus et al.[12], the effect size was determined as d=0.55. With 80% power and 0.05 alpha, the minimum sample size to be included in the study was calculated as n=88: a control group of n=35 and a patient group of n=53. Data were expressed as mean±standard deviation or median, 25th percentile-75th percentile, frequency and percentage. Independent sample t test was used to compare continuous normal data between groups. Mann Whitney U test was used to compare continuous non-normal data between groups. Categorical variables were expressed as numerical or percentage. Pearson or spearman correlation coefficient were used for correlation between variables. Receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic performance of niacin and DA in FMS. P values were considered statistically significant when calculated less than 0.05. The analyzes were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., An IBM Co., Somers, NY).

RESULTS

Mean age of patients and healthy controls were 38.34±5.5 and 36.72±5.19 respectively. Body Mass Indexes (BMI) of patients and controls were 29.35±5.01 and 27.82±4.33 respectively. There was no significant difference between patient and control groups in terms of age and Body Mass Index values (p=0.17 and p=0.14 respectively). FMS patient group mean Vit C values [0.40 mg/dl (0.14-0.66 mg/dl)] were determined to be significantly lower than those of controls [0.56 mg/dl (0.30-0.82 mg/dl) (p: 0.004)]. Similarly, mean TAC values of the patient group [0.84mmol/L (0.69-0.99)] were figured out to be significantly lower than those of controls [0.95 mmol/L (0.71-1.19) (p: 0.009)]. For OSI, patient group mean values [1.16 (0.88-1.44)] were significantly lower than control group values [1.03 (0.87-1.29)] (p: 0.048) and distribution of variables by groups were shown in **Table 1**.

FMS patients self reported considerably high scores of VAS; 7.88±1.84 (0-10), FIQ; 65.12±12.7 (0-100) and TPN; 13.46±2.7 (0-18) and a moderate score of BDI; 23.34±10.3 (0-63). Distribution of quantitative variables of FMS Patients were shown in **Table 2**.

Table 1. Distribution of variables by groups

Variables	FMS Patient Group (n=53)	Control Group (n=35)	p
Age (Years)	38.34±5.5	36.72±5.19	0.17*
BMI(kg/m²)	29.35±5.01	27.82±4.33	0.14*
TOS	9.7±1.97	9.3±1.71	0.333
TAC	0.84±0.15	0.95±0.24	0.009
OSI	1.16±0.28	1.03±0.26	0.048
Vit C	0.40±0.26	0.56±0.26	0.004

Values are presented as median (25%-75%) percentiles *Independent samples t test was used. **: Mann Whitney U test was used. BMI: Body Mass Index, DA: Dopamine, kg/m²: kilogram/square metre, ng/mL: nanogram/mililitre, nmol/L:nanomol/Litre.

Table 2. Distribution of quantitative variables of FMS Patients

Variables	Mean±SD (n=53)	Minimum	Maximum
VAS	7.88±1.84	0	10
TPN	13.46±2.7	0	18
BDI	23.34±10.3	0	63
FIQ	65.12±12.7	0	100

Values are presented as Mean±SD, BMI: Body Mass Index, VAS: Visual Analogue Scale, TPN: Tender Point Numbers, BDI: Beck Depression Inventory, FIQ: Fibromyalgia Impact Questionnaire.

In patient group, correlation analysis between FIQ and TPN levels gave a moderate positive correlation (r=0.505; p <0.001). Bivariate correlation of quantitative variables are shown in **Table 3**.

Table 3. Bivariate correlation of quantitative variables

		Tender point	FIQ	OSİ	TAC
Tender Point	r	1	0,505*	-0,084	0,237
	p		<0,001	0,560	0,098
FIQ	r	0,505*	1	-0,191	-0,007
	p	<0,001		0,190	0,962
OSİ	r	-0,084	-0,191	1	-0,130
	p	0,560	0,190		0,234
TAC	r	0,237	-0,007	-0,130	1
	p	0,098	0,962	0,234	
VitC	r	0,143	0,246	0,148	0,105
	p	0,322	0,088	0,182	0,334

Pearson correlation coefficient was used. *: 0,05 significance level

When we evaluate the diagnostic performance of VitC and TAC by ROC analysis, cutoff values of ≤0.350 and ≤0.924 were found respectively. ROC analysis and their curves of TAC and Vit C are seen in **Table 4** and **Figure 1** respectively.

Table 4. The ROC analysis of Vit C and TAC

Variable	Cutoff	AUC	Sensitivity	Specifity	PPV	NPV	p
Vit C	<=0.350	0.678	0.528	0.788	0.790	0.525	0.003
TAC	<=0.924	0.639	0.774	0.486	0.695	0.586	0.028

AUC: Area under curve; Se: Sensitivity; Sp: Specifity; PPV: Positive predictive value; NPV: Negative predictive value, kg/m²: kilogram/square metre, ng/mL: nanogram/mililitre, nmol/L:nanomol/Litre.

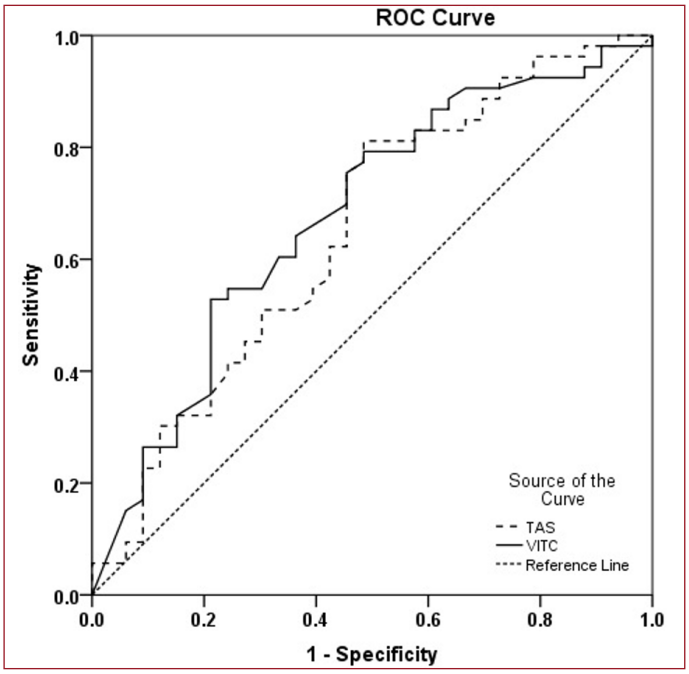

Figure 1: ROC Analysis of TAS, and Vit C

DISCUSSION

In our study serum TAC and vitamin C levels were found to be low and OSI was high indicating that FMS is an over-oxidative state that is not adequately met by antioxidants for various reasons. In line our results there have been some studies. Bengtsson et al.[13] assessed metabolism of oxidation in FMS patients and determined that levels of adenosine monophosphatase and creatinine increased while adenosine diphosphatase and creatinine phosphate levels decreased. Bagis et al.[14] defined that the levels of plasma lipid preoxidaation (LP) of active FMS patients were much more than controls. Lately published article showed that plasma TAC level of FMS patients is significantly lower than those of control group. In FMS patients, increased TOS and OSI have been reported by Neyal et al.[15] Acute phase reactants like WBC, CRP, ESR etc. are main markers that can help diagnose and determine the effectiveness of medical treatment of inflammatory conditions. In blood mononuclear cells (BMCs), bioenergetic status, oxidative stress and their connection with headache symptom in FMS were studied by Cordero et al.[16] In addition, they evaluated the effects of oral coenzyme Q10 (CoQ10) supplementation on biochemical markers and clinical recovery. They found a decrease in CoQ10, catalase and ATP levels in FMS patients compared to normal controls (P <0.05 and P <0.001, respectively). In FMS patients, it was found higher levels of LPO in BMCs than controls (P <0.001). Significant negative correlations were observed between CoQ10 or catalase levels and headache symptoms in BMCs (r=20.59, P <0.05; r=20.68, P <0.05, respectively). In addition, there was a significant positive correlation of LP levels with headache impact test (HIT-6) (r=0.33, P <0.05). Supplementation of oral CoQ10 corrected biochemical parameters and caused significant improvement in clinical and headache symptoms (P <0.001). Bozkurt et al.[17] also investigated oxidative stress and prolidase enzyme activity in FMS patients considering body mass index (BMI), serum TAC or paraoxonase-1 (PON-1) levels and did not find any difference comparing to the controls. However, regarding TOS and OSI with high serum prolidase activity there was a clear difference between FMS patients and controls. Serum TOS, OSI, VAS and fatigue scores were positively related with serum prolidase activity. In our study, in accordance with the existing literature, TAC levels were discovered to be significantly lower in the patients compared to the controls (p: 0.009). In parallel, OSI was significantly higher in the patient group than those of controls (p: 0.048). Cordero et al.[18] found that in FMS pathophysiology inflammation has a role and that is caused by unmet oxidative stress and mitochondria dysfunction. Another study showed that the levels of pro-inflammatory cytokines increased in serum and biopsy specimens of FMS patients. Between plasma LP, total FIQ score and VAS for clinical symptoms in FMS patients a positive correlation was identified. In contrast to the above mentioned studies, Bozkurt et al.[17] showed that the acute phase reactants levels did not differ between FMS patients and controls.

In our study, there is a moderate, positive and significant correlation between the tender point numbers and FIQ, which is compatible with the previous studies (r=0.505; p <0.001). In their study, Eisinger et al.[18] although protein peroxidation could be demonstrated in patients with FMS, they could not show a difference in LP and MDA levels between patients and controls. Although Fassbender and Wegner[19] stated that local hypoxia of muscles causes tender points of muscle. Lund et al.[20] stated that abnormal oxygen pressure is seen on the surface of the muscle above the trigger points. In the study of Naziroglu et al.[21] LP levels in plasma and erythrocytes were found to be lower in patients comparing controls, while LP levels in vitamin C and E (VCE) and exercise groups were lower than baseline levels after 12 weeks. Plasma concentrations of vitamins A, E and reduced glutathione were lower in patients compared to the controls. VCE and exercise increase their concentrations. In erythrocytes, VCE supplementation with or without exercise increases glutathione peroxidase activity. In all groups, treatment can't change β-carotene concentrations. However treatment has the measured effects on anti-

oxidative mechanisms, FMS symptoms did not improve. FMS-induced oxidative stress can be prevented by exercise and VCE by up-regulation of an antioxidant redox system. They concluded that the combination of protective effects of VCE with exercise was greater than just exercise.

In our study, Vit C levels were measured to be significantly lower in the patient group (p: 0.004). Joustra et al.[22] in their study, compared patients in chronic fatigue syndrome (QMS) and FMS with controls, to examine the relationship between mineral, vitamin levels and clinical manifestations, including the severity of symptoms, life quality, and the results of supplementation on clinical findings. It has been searched in EMBASE, PubMed, PsycINFO and Web of Knowledge databases. Patients in the articles published from 1990 for FMS to 1 March 2017 were evaluated. Articles are included in the study if one or more vitamin or mineral states are given or if a supplemantation with minerals or vitamins has been made. Circulating vitamin E concentrations were lesser in patients than in controls (aggregated standard mean difference (SMD):-1.57, 95% CI: -3.09, -0.05; p=.042). But there was no difference when limiting the analysis to the high quality scores subgroup. There was no any repeatedly or continuously connection between clinical parameters and minerals or vitamins. Also, randomized controlled trial (RCT) tests including these mineral and/or vitamins supplements did not give improvement of clinical symptoms.

Our study has some limitations; the diarrhea causing Vitamin C deficiency could not be ruled out. TPN measurements were could be performed with algometer instead of manual finger pressure method. TAC levels could only be measured in serum, but they could also be measured in CSF and urine samples. Since our studyis cross-sectional, it could not say anything aboutthe effects replacement of deficient vitamin.

In our study, Vit C and TAC distinguished patients and controls with 0.350 mg/dl cutoff value 52.8% sensitivity and 78.8% specificity and 0.924 mmol/L cutoff value 77.4% sensitivity and 48.6% specificity, respectively.

CONCLUSION

The results of this study showed us that serum vit C and TAC levels decreased and OSI increased in FMS patients compared to controls. Oxidative stress is included in FMS pathophysiology causing pain in muscle, stiffness and tenderness in tendons and joints. The absence of specific anatomical, histological or molecular markers of the disease makes diagnosis difficult. It was thought that decrease of TAC levels and increase of OSI may be due to insufficient vit C intake. It can be suggested that these three parameters can be considered as an additional tool for diagnosis of FMS. It is believed that monitoring of serum vit C, TAC and OSI levels in FMS patients and treatment for this will improve the prognosis of the disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: Tokat Gaziosmanpasa University School of Medicine Ethics Committee of Clinical Reseaches approved our study with the code of 20-KAEK-030 at 06.02.2020.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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