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Research Article

An Evaluation of the Plasma Free Amino Acid Profile Level and Ultrasound Elastography Data of Patients with Fibromyalgia

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ABSTRACT

Objectives: The aim of this study was to clarify the relationship of the pathogenesis of widespread pain and the measured levels of plasma free amino acids in patients with fibromyalgia syndrome, and to evaluate the relationship with symptom severity. It was also aimed to examine the effect of plasma amino acid profile levels on muscle elasticity by evaluating potential tissue changes in trapezius and rhomboid muscles with ultrasound elastography.

Patients and methods: Fifty female patients with FMS and 47 healthy women were included in the study. The severity of pain was measured with a Visual Analog Scale, and the patients completed the Fibromyalgia Impact Questionnaire to determine functional status. Blood samples were taken from all the patients and control subjects. All groups were evaluated with ultrasound elastography.

Results: The plasma cysteine, glutamine, glycine, serine, ethanolamine, norvaline and argininosuccinic acid levels were determined to be statistically significant higher in the patient group than in the control group. The anserine, ortho-phosphorylethanolamine, and cystathionine levels were determined to be statistically significantly lower in the patient group than in the control group. The right and left rhomboid major muscles stiffness of the patient group were found to be statistically significantly higher than those of the control group.

Conclusion: In conclusion, some amino acid levels, there could be a relationship between the amino acid changes and pain severity. There was found to be increased stiffness in the rhomboid muscles of the FMS patients compared to the healthy control group.

Keywords: Fibromyalgia; Amino acid; Elastography

INTRODUCTION

Fibromyalgia Syndrome (FMS) is a chronic syndrome of unknown etiology, characterised by widespread pain in the musculoskeletal system and sensitivity in specific anatomic regions, accompanied by sleep disorders, fatigue, cognitive function disorders, depression, anxiety, and several somatic symptoms [1]. Although FMS is a disease diagnosed in both genders and all age groups, it is seen most often between the ages of 20-50 years. It is seen in females 4-9-fold more than in males [2,3].

The aim of this study was to clarify the relationship of the pathogenesis of widespread pain and the measured levels of plasma free amino acids in patients with fibromyalgia syndrome, and to evaluate the relationship with symptom severity. It was also aimed to examine the effect of plasma amino acid profile levels on muscle elasticity by evaluating potential tissue changes in trapezius and rhomboid muscles with Ultrasound Elastography (UE). Thus, the importance of the amino acid profile in the treatment of the disease will be determined by the diagnosis and/or etiology of FMS.

MATERIALS AND METHODS

Approval for this study was granted by the Clinical Research Local Ethics Committee (decision no:19/10/53, dated 09.09.2019). Informed consent was obtained from all the patients and healthy control subjects included in the study.

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The study included a total of 50 female patients who presented as outpatients with complaints of widespread pain and were diagnosed with FMS (newly diagnosed and those with known FMS). Inclusion criteria were age between 18 and 55 years, meeting the 2010 ACR fibromyalgia diagnostic criteria, and agreement to participate in the study. Patients were excluded from the study if they had a history of systemic disease (hypertension, diabetes, cardiovascular, renal diseases), a history of malignancy, the presence of active infection, neuromuscular diseases, autoimmune diseases, if they were pregnant or breastfeeding, if they had a history of psychiatric disease, or a history of surgery in the back region where UE was to be applied.

The control group was formed of 47 age-matched healthy females, with no chronic disease and no diagnosis of FMS.

In the first examination of the FMS patients, the severity of pain was measured with a Visual Analog Scale (VAS), and the patients completed the Fibromyalgia Impact Questionnaire (FIQ) to determine functional status. The VAS used to determine the severity of pain in the patient group is an easily applicable scale, accepted and used throughout the world to measure pain levels. The evaluation of pain, which is a subjective feeling, is rendered into numerical form and thus the severity is determined. The patient is instructed to mark the level of pain felt on a 10cm line marked in cm where 0= no pain and 10=intolerable pain [4,5]. In this study pain severity was classified as 0-3 points: mild, 4-6 points: moderate, and 7-10 points: severe [6].

The FIQ was used in the current study to determine the functional status and quality of life of the FMS patients. The FIQ, which is widely used in the evaluation of FMS patients, was developed by Yelin et al. and the reliability and validity studies of the Turkish version [7]. The scale comprises 10 items, the first of which is scored from 0-3 points (0: never, 1: usually, 2: sometimes, 3: never) for each of 11 daily living activities (shopping, cooking, washing up, vacuuming, making beds, walking several hundred metres, social visiting, gardening, driving, going up and down stairs). The other items question how the patient has felt in the last week in respect of feeling well, inability to work, pain level, fatigue, morning stiffness, morning tiredness, anxiety and depression. The 11 daily activities in the first question are scored from 0-3, and the total of these is divided by 11 then multiplied by 3.33 to normalise the value. In the second item, as the number of days that the patient has felt well is in reverse proportion to the disease severity, the number of days specified by the patient is subtracted from 7 then multiplied by 1.43. In item 3, the number of days on which the patient could not work is multiplied by 1.43 to normalise the value. The total score for the whole FIQ is then obtained by adding the totals indicated for items 4-10 to the normalised values of items 1-3. The maximum FIQ score is 100 with higher points indicating a low functional status and quality of life.

Blood samples were taken from all the patients and control subjects after an 8-hour fasting period into EDTA tubes. The samples were transferred on ice to the biochemistry laboratory. The gel tubes were centrifuged at 5000 rpm for 10 mins, then using a pipette, the separated serums were placed in Eppendorf tubes. The tubes were numbered corresponding to the patient names in both groups and the samples were stored at -80°C until assay.

For examination of free amino acids, the samples were thawed back to room temperature and the analysis was performed using the JASEM amino acid kit.

The patients and control subjects were evaluated with UE. The examination was made in the semi-dark US room at 25°C, with the subject in a prone position, and using a Siemens ACUSON S3000 device with shear-wave elastography software with a 9L4 linear probe. For imaging of the muscles, first B-mode imaging was applied, then elastography synchronised with the B-mode method.

By forming 4 different colour-coded quantitative and qualitative tissue maps (shear wave velocity, quality control, tissue movement, shear wave duration), the probe was placed on 3 separate points in the longitudinal plane on the left and right of the upper trapezius muscle and the rhomboid major muscle, values were obtained as m/sn and kPA, and the mean value of these was used for analysis.

Data obtained in the study were analyzed statistically using SPSS vn 22.0 software. Conformity of the data to normal distribution was assessed with the Kolmogorov-Smirnov test. In the evaluation of two independent groups of data not showing normal distribution, the Mann Whitney U-test was applied and for more than two independent groups, the Kruskal Wallis test. Data conforming to nomal distribution were compared with the t-test. A value of $p<0.05$ was accepted as statisticaly significant.

RESULTS

The mean age of the patients was 38.94 ± 8.94 years (range, 23-55 years) in the patient group and 37.28 ± 9.06 years (range, 22-55 years) in the control group. No statistically significant difference was determined between the groups in respect of age ($p>0.05$). Marital status was recorded as married in 41 (82%) and single in 9(18%) patients in the FMS group and married in 34 (72.3%) and single in 13 (27.6%) subjects in the control group, with no significant difference determined between the groups ($p>0.05$).

The VAS score of the patient group was mean 7.18 ± 1.53 (range, 3-10), and the FIQ score was mean 66.64 ± 11.89 (range, 32-89).

When the plasma-free amino acid levels of the patient and control groups were examined, the plasma cysteine ($p=0.000$), glutamine ($p=0.030$), glycine ($p=0.000$), serine ($p=0.024$), ethanolamine ($p=0.027$), norvaline ($p=0.000$) and argininosuccinic acid ($p=0.001$) levels were determined to be statistically significant higher in the patient group than in the control group. The anserine ($p=0.014$) ortho-phosphorylethanolamine ($p=0.014$), and cystathionine levels ($p=0.048$) were determined to be statistically significantly lower in the patient group than in the control group. In the other amino acids examined, no statistically significant difference was observed between the groups ($p>0.05$).

The right and left trapezius and major rhomboid muscles of the patients and control group subjects were evaluated with shear-wave elastography. For each muscle, elasticity as kPa value and shear wave velocity as n/sn were measured 3 times and the mean values were calculated (Emean and Vmean). The Emean and Vmean values of the right and left rhomboid major muscles of the patient group were found to be statistically significantly higher than those of the control group ($p<0.005$). No difference was determined between the groups in respect of the trapezius muscle values (Tables 1 and 2).

The patient group was subdivided according to the VAS scores

Table 1: Analysis of shear wave elastography data in terms of elasticity (kPa).

	Patient (n:50)			Control (n:47)			p
	Median	Min.	Maks.	Median	Min.	Maks.	
RTEmean. (kPa)	28.25	14.7	77.77	32.57	12.73	77.83	0.244
LT Emean. (kPa)	25.56	14.67	58.5	27	13.93	63.27	0.705
RREmean. (kPa)	67.03	25.73	172.9	46.3	20.53	118.97	0
LREmean. (kPa)	58.48	18.53	104.03	39.73	22.97	108.67	0

Note: RT:Right Trapez; LT:Left Trapez; RR: Right Rhomboid; LR:Left Rhomboid

Table 2: Statistical analysis of free amino acids according to VAS values among the patient group.

	VAS-1 (n:2)			VAS-2 (n:10)			VAS-3 (n:38)			p
	Median	Min.	Maks.	Median	Min.	Maks.	Median	Min.	Maks.	(<0.05)
Alanin	743.21	688.17	798.25	609.22	248.62	1001.91	436.11	198.47	603.39	**0.009**
Ornitin	202.4	181.55	223.25	169.6	91.18	250.12	115.14	45.93	244.17	**0.038**
Tireonin	206.5	105.62	307.38	156.92	54.55	307.38	100.83	43.67	190	**0.011**
Valin	389.98	353.35	426.61	343.28	223.71	509.99	290.5	80.81	545.76	**0.039**
Sarkozin	847.87	841.25	854.49	606.92	332.42	1165.01	440.29	232.56	612.05	**0.003**

for pain severity as mild (n:2, VAS1), moderate (n:10, VAS2), and severe (n:38, VAS3). When the plasma amino acid values were examined according to the subgroups of VAS score, the plasma alanine (p=0.009), ornithine (p=0.038), threonine (p=0, 011), valine (p=0.039), and sarcosine (p=0.003) levels of the VAS1 group with a mild level of pain were determined to be statistically significantly higher than those of the other groups. No significant difference was determined between these groups in respect of the shear-wave elastography data.

DISCUSSION

In this study, it was aimed to determine whether or not these markers could be used as a quantitative method in the diagnosis of FMS, and to determine the relationship with symptoms and the importance of plasma free amino acids in treatment of the disease.

The serum tryptophan and other amino acid levels were investigated. Tryptophan is a precursor of serotonin and is an amino acid that can pass the blood-brain barrier. Serotonin is a neurotransmitter responsible for the perception of pain, appetite, mood, libido, cognitive state, and especially the modulation of non-REM sleep. Pain modulation in the central and peripheral nervous system is corrected by decreasing the substance P levels effective in the formation of pain. Serine, histidine, arginine, methionine and threonine levels and primarily the serum tryptophan value have been determined to be lower in FMS patients compared to a control group [8].

Serotonin and plasma branched chain amino acid concentrations (valine, leucine and isoleucine) were examined and levels of phenylalanine, valine, leucine, isoleucine and tryptophan were found to be lower in patients with fibromyalgia than in control subjects. It was reported that this decrease in branched chain amino acids could theoretically lead to a decrease in muscle protein synthesis causing the loss of muscle energy [9].

The concentrations of 20 amino acids in 34 patients affected by fibromyalgia and 18 control subjects. Plasma taurine, alanine, tyrosine, valine, methionine, phenylalanine and threonine concentrations were determined to be significantly lower in the patient group than the control group [10]. FMS patients and 20 healthy females, serum aspartate, glutamate, glycine, isoleucine, leucine, methionine, ornitine, phenylalanine, sarcosine, serine, taurine, tyrosine, and valine concentrations were found to be high in the patient group compared to the control group. Increased glutamine- glutamate levels increase the formation of free radicals by causing an increase in glutamate receptor activation. This was reported to cause a worsening of quality of life in patients [11]. In the current study, the plasma cysteine, glutamine, glycine, serine, ethanolamine, norvaline, ortho-phosphorylethanolamine, and arginosuccinic acid concentrations were determined to be statistically significantly high in the FMS patients, and the plasma cystathionine and anserine concentrations were found to be significantly low. The plasma glutamine, as reported in the study by Ruggiero et al. and the plasma glycine and serine amino acids, were determined at a higher concentration in the current study FMS patients than in the control group. Glutamine is the most abundant free amino acid found in human plasma and muscles, which is formed by the breakdown of glutamate with its nitrogen molecule and plays a role in the regulation of MSS functions by participating in glutamate and GABA synthesis. Previous studies have determined a correlation between serum glutamate concentrations and CSF levels [12]. Glutamate and glutamine values are increased in the brain of FMS patients and it has been reported that this increase could be related to the increase in pain sensation [13].

Glycine is a significant neurotransmitter in central sensitisation which shows an effect as a positive modulator of N-methyl-D-aspartic-acid (NMDA) receptor together with glutamate [14]. This increased glycine concentration helps to explain the increase in pain sensation in FMS patients [15,16]. The reason that unlike some other studies in literature a high serine concentration was determined in the current study, can be considered to be that there could be a relationship between the serine amino acid, which is a precursor of glycine, and NMDA receptor activity, which is effective in pain sensitivity [17].

In the current study patient group, a low concentration was determined of anserine, which is an anti-inflammatory dipeptide formed from β-alanine and 1-methyl histidine [18]. Anserine is a natural derivative of carnosine (β-alanine) not found in human tissues and fluid so must be taken in with the diet. Anserine and carnosine have common physiological functions such as anti-oxidation, acid base balance and metallic chelation [19,20]. In addition, the activity of calcium channels in skeletal muscles is regulated [21]. Mathiowetz et al. examined the effect of anserine and carnosine supplementation on cognitive function and physical activity in the elderly. In the group given anserine and carnosine supplementation for 3 months, there was determined to be an increase in physical capacity and in cognitive functions as an abstract concept [22]. In a study by Culver et al. it was observed that long-term beta alanine supplementation increased carnosine concentration in skeletal muscles and there was also an increase in anaerobic short-term exercise capacity [23]. Consequently, when the studies in literature are taken into consideraton, the low anserine level determined in the current study patient group may help to explain the worsening of pain and cognitive functions in FMS patients.

In the majority of studies in literature, a decrease has been determined in alanine, threonine, and valine concentrations in FMS groups compared to control groups [9,24]. Unlike previous studies, no statistically significant difference was determined between the patient and control groups in respect of these amino acids, and when patients were evaluated in respect of pain severity, there was determined to be a significant decrease in these amino acid concentrations as the VAS score increased. Alanine, which has a role in trytophan metabolism, is an amino acid which prevents the accumulation of lactic acid in the muscles during exercise by increasing carnosine synthesis together with anserine (β-alanine histidine) [25]. Thus, by providing energy during intense physical activity, damage to the muscles and central nervous system is prevented [26]. Valine increases glucose entry to the muscle cells by stimulating insulin synthesis during physical activity and provides energy to the muscles, and therefore by preventing muscle damage, a contribution is provided to regeneration [27]. When these factors are taken into consideration, it can be understood that the decrease in amino acid levels caused an increase in pain severity and muscle fatigue in patients. It was thought that as the pain severity increased in the current study patients, the reduction in amino acid concntrations was associated with this mechanism.

Elasticity refers to the ability of a tissue to be deformed under pressure and to return to the previous form when the pressure is removed. Muscle stiffness measurements applied correctly and in real time provide support in diagnosis, treatment and follow up of acute and chronic musculoskeletal system pathologies such as chronic myofascial pain with acute musculoskeletal system injuries [28]. Although muscle biopsies provide detailed information about the microscopic structure of the muscle, it is not a useful method in screening programs as it is an invasive procedure. However, UE has an important place in the diagnosis and treatment of pathologies by providing real-time and accurate measurements without any effects of restrictions [29,30]. Measure blood flow in sensitive points of FMS patients and investigated the relationship between these flow velocities and disease symptoms and quality of life. That study included 24 female FMS patients with sensitive points in the upper part of the trapezius muscle and in the supraspinatus muscle, and blood flow was measured with UE. A positive correlation was determined between UE flow velocities in the right trapezius neutral position and some quality of life scores (SF-36) and between UE flow velocities in the left trapezius neutral position and VAS scores. It was concluded in that study that to be able to use UE flow velocity values as a quantitative diagnostic criteria in FMS patients, there was a need for further studies with a greater number of patients and a control group [31].

In the current study, in addition to considering the VAS score of pain severity in the patient group was compared with UE flow velocities. However, no significant relationship was determined between the VAS score and flow velocities. The reason for this could be factors such as the fact that pain is subjective, different patients have different pain thresholds, and the thoughts of patients that there could be a secondary benefit associated with the pain severity.

CONCLUSION

In conclusion, in addition to the differences determined between the patients diagnosed with fibromyalgia and the healthy control group in respect of some amino acid levels, there could be a relationship between the amino acid changes and pain severity. Just as this may explain FMS etiopathogenesis and clinical symptoms, the dietary supplementation of amino acids determined to be deficient in the patient group could have an effective role in the treatment of FMS.

In the current study, there was found to be increased stiffness in the rhomboid muscles of the FMS patients compared to the healthy control group. When the importance that ultrasonographic examinations have acquired in musculoskeletal system problems in recent years is considered, the SWE method can be considered for use in the diagnosis and treatment of FMS patients to be able to obtain quantitative data and visualise changes in the muscle structures which are thought to be effective in the muscle pain which is the primary complaint of FMS patients. Nevertheless for the changes in amino acid levels and shear-wave elastography to be able to be used routinely in the diagnosis and follow up of FMS, there is a need for further studies with a greater number of patients and control groups.

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