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The relationship between serum prolidase activity and histone H3 protein levels and fibromyalgia

A. OZTURK[1], T. AGBEKTAS[2], A. TAS[3], M.A. GUL[4], A. KARADAG[5], Y. SILIG[2]

[1]Clinical Biochemistry, Health Sciences University, Etlik City Hospital, Ankara, Turkey
[2]Department of Medical Biochemistry, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[3]Department of Nutrition and Dietetics, Faculty of Health Sciences, Sivas Cumhuriyet University, Sivas, Turkey
[4]Department of Medical Biochemistry, Faculty of Medicine, Amasya University, Amasya, Turkey
[5]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey

Abstract. – OBJECTIVE: Fibromyalgia (FM) is a clinical syndrome characterized by prominent physical and psychological impairment and widespread pain on both sides of the body, above and below the waist, and along the axial skeleton. It often causes sleep difficulties, memory impairment, mood changes, irritable bowel syndrome, and fatigue. Our study aimed to investigate the relationship between FM and prolidase (peptidase D) and histone H3 protein levels by comparing a patient group with a healthy control group.

PATIENTS AND METHODS: In total, 176 people were examined in our study, 88 of whom were healthy and 88 of whom had FM. Serum level was measured by ELISA. Then the results were analyzed using SPSS. All $p < 0.05$ were considered statistically significant.

RESULTS: A significant increase in the levels of prolidase was observed in the patient group compared with the control group (6.28-4.68, $p < 0.001$). Histone H3 protein values were not significantly different between the patient and control groups ($p=0.184$). The ROC analysis indicated that prolidase was statistically significant in disease prediction ($p<0.001$, AUC: 0.795 (0.697-0.893), while histone H3 protein was statistically insignificant in predicting disease.

CONCLUSIONS: The results of the study show that prolidase activity may play a role in diagnosing FM. In addition, since no study like ours has been performed before, it can bring a new perspective to the literature.

Key Words:
Fibromyalgia, Prolidase activity, Histone H3 protein.

Introduction

Fibromyalgia (FM) is a chronic musculoskeletal syndrome characterized by widespread pain, fatigue, stiffness, and tenderness in certain anatomical areas called "tender points"[1-3]. There is no well-defined organic disease underlying FM. Joint stiffness, chronic pain at multiple tender points, cognitive dysfunction, sleep problems, anxiety, depression, and fatigue are seen[4,5]. FM is a heterogeneous syndrome associated with various diseases such as infections, psychiatric or neurological disorders, diabetes, and rheumatoid pathologies. This disease is more common in women, significantly reduces the quality of life, and is costly in terms of social and health care. For treatment, a patient-specific plan is usually required, and pharmacological treatment is applied by analyzing the risk-benefit ratio[6,7]. The prevalence of FM, the third most common diagnosis in rheumatology clinics, varies between 1.3% and 8% in the general population[5]. There are no specific tests for the diagnosis of FM. It is currently recognized using a diffuse pain index (dividing the body into 19 regions and scoring how many regions are reported as painful) and a symptom severity score (SSS), which assesses cognitive symptoms[8]. The mechanism of the emergence of FM is not very clear.

Changes in collagen metabolism contribute to FM pathogenesis. Nerve endings at tender points have a distinctive histological appearance containing layers of the organized collagen matrix, which may indicate abnormal collagen metabolism at these sites. Specifically, muscle biopsies from FM patients show a characteristic collagen cuff surrounding axons, which is associated with myofibril disorganization and contributes to post-exercise pain and stiffness[9]. The authors of a previous study suggested that understanding collagen metabolism in FM may be central to understanding its etiology[10].

Corresponding Author: Alpaslan Ozturk, MD; e-mail: dralpaslanozturk@gmail.com

Prolidase (peptidase D), a member of the matrix metalloproteinase family, is a cytosolic exopeptidase that specifically cleaves imidodipeptides containing proline and hydroxyproline at the C-terminus. This enzyme plays an important role in collagen metabolism, matrix remodeling, and cell growth[11]. In addition to plasma, it is found in leukocytes, erythrocytes, and fibroblasts[12]. It has also been shown to play a role in inflammation-related physiological and pathological processes such as wound healing and cell migration[13]. There is only one study[14] in the literature investigating the serum prolidase activity in FM patients, and it was reported that increased prolidase activity may contribute to the pathogenesis of FM and measuring serum prolidase enzyme activity could be a useful FM biomarker. The relationship between serum prolidase activity and many diseases such as benign prostatic hyperplasia[15], pediatric bipolar disorder[16], subclinical vascular damage in children with epilepsy[17], isolated coronary artery ectasia[18], polycystic ovary syndrome[19], gastric cancer[20], psychosis[21], myocardial infarction[22], preeclampsia[23], infertility[24], obesity[25], Alzheimer's disease[26], osteoarthritis[27], peripheral vertigo[28], bladder cancer[29], aphthous stomatitis[30], and familial Mediterranean fever[31] has been investigated and shown so far.

Studies[32-35] show that neutrophil extracellular traps (NETs) may play a role in the pathogenesis of various diseases, such as sepsis, asthma, rheumatoid arthritis, periodontitis, and malignant neoplasia. Extracellular traps are net-like structures covered with histone granular and cytosolic proteins formed by various immune system cells, including neutrophils, eosinophils, and macrophages[36]. The effect and presence of the formation of extracellular traps in FM patients have not yet been extensively studied. One of the main reactions in the formation of NETs is histone citrullination by the histone-specific enzyme peptidyl deiminase-4[37]. Studies[38-40] have shown that histone H3 protein (H3cit) is a specific marker in the formation of extracellular traps and can be detected in tissue samples. Histone H3 protein can also be analyzed in peripheral blood samples[41-44]. To the best of our knowledge, there is no study investigating the relationship between serum prolidase activity and histone H3 protein in FM patients. The aim in the present study was to investigate serum prolidase enzyme activity and histone H3 protein levels, a specific marker of neutrophil extracellular traps, in FM patients.

Patients and Methods

Patients

The patient group consisted of 88 patients who were diagnosed with FM in the Department of Physical Medicine and Rehabilitation Clinic at Cumhuriyet University's Faculty of Medicine Research and Application Hospital. The study was conducted on serum samples collected between September 2018 and December 2019. Individuals newly diagnosed with FM according to the criteria of the American College of Rheumatology[45] were included in the study.

The exclusion criteria for FM patients were: inflammatory rheumatic disease (lupus, rheumatoid arthritis, seronegative spondyloarthritis, Behçet's disease, etc.), malignancy, a known systemic disease history (heart, endocrine, psychiatric, neurological disease, etc.), regional pain syndrome (low back pain, osteoarthritis, myofascial pain, etc.), or any medication other than simple analgesics.

Controls

The control group consisted of 88 healthy individuals who applied to Cumhuriyet University Hospital, who had not been diagnosed with FM and chronic diseases before, and who showed a similar distribution in terms of sex and age with the patient group. The study was conducted using serum samples obtained from blood collected with the permission of the Ethics Committee with the decision, Approval Number: 2022-01/34 (Dated: 13.01.2022).

Measurement of Serum Prolidase and Histone H3 Levels

Written informed consent was obtained from all who participated in the study. It was conducted in accordance with the ethical standards of the Declaration of Helsinki on human experimentation. The aim of our work was explained to the participants and a written informed consent form was signed by all.

In the first phase of our work, blood samples were placed in 8-mL serum tubes with citrate and clot activator. Afterward, the blood samples were centrifuged at 1,000 rpm for 15 minutes to obtain serum and plasma samples. The serum samples obtained were stored for later use in the study.

Serum prolidase (ELK Biotechnology, Cat.: ELK6673) and histone H3 (ELK Biotechnology, Cat: ELK8743) levels were measured using a commercially available human enzyme-linked immunosorbent assay (ELISA) kit according to

the manufacturer's instructions. The detectable maximum and minimum value ranges of the prolidase and histone H3 ELISA kits were 1.57-100 ng/mL. The minimum measurable levels for serum prolidase and histone H3 were 0.63 ng/mL and 0.62 ng/mL, respectively.

Statistical Analysis

The statistical analysis was carried out using SPSS Statistics Version 22 (IBM Corp., Armonk, NY, USA). All demographic characteristics and categorical data were presented as frequencies (n) and percentages (%). The Chi-square test was used to analyze the ratio comparison between the patient and control groups. Descriptive statistics were presented as mean±standard deviation (SD) for normally distributed numerical data and median (quartiles) for non-normally distributed numerical data. The Shapiro-Wilk test was used to determine whether the data had a normal distribution. The relationships between the numerical variables were analyzed using the Spearman correlation coefficient as the data were not normally distributed. Student's *t*-test was used for normally distributed data and the Mann-Whitney U test for non-normally distributed data to compare numerical data between the patient and control groups. Receiver operating characteristic (ROC) analysis was used to distinguish the patient group from the control group using prolidase values. The area under the ROC curve (AUC) with 95% confidence intervals was calculated. AUC was assessed as follows: 0.9-1 excellent, 0.8-0.9 good, 0.7-0.8 fair, 0.6-0.7 poor, and 0.5-0.6 very poor. Following the ROC analysis, the Youden index (maximum sensitivity and specificity) was used to determine the best cut-off point for the prolidase values found significant in the ROC analysis. $p<0.05$ was chosen as the statistical significance level.

Results

A total of 176 participants, 88 patients and 88 controls, were analyzed in the study. Statistical findings for the comparison of sociodemographic and clinical characteristics between the research groups are presented in Table I. The sex distribution was not significantly different between the groups ($p=0.550$). While 8% (n=7) of the patient group were male and 92% (n=81) were female, 5.7% (n=5) of the control group were male and 94.3% (n=83) were female. The average age was

Table I. Comparison of sociodemographic and clinical values between the patient and control groups.

		Patient (n = 88)	Control (n = 88)	*p*-values
Sex	Male	7 (8%)	5 (5.7%)	0.550[a]
	Female	81 (92%)	83 (94.3%)	
Age		43.94 ± 8.33	43.18 ± 5.56	0.477[c]
Sleep disorder	Yes	69 (78.4%)	36 (40.9%)	< 0.001[a]
	No	19 (21.6%)	52 (59.1%)	
Fatigue	Yes	84 (95.5%)	66 (75%)	< 0.001[a]
	No	4 (4.5%)	22 (25%)	
Headache	Yes	81 (92%)	46 (52.3%)	< 0.001[a]
	No	7 (8%)	42 (47.7%)	
Morning fatigue	Yes	83 (94.3%)	47 (53.4%)	< 0.001[a]
	No	5 (5.7%)	41 (46.6%)	
Dry mouth	Yes	69 (78.4%)	44 (50%)	< 0.001[a]
	No	19 (21.6%)	44 (50%)	
Leg Numbness	Yes	72 (81.8%)	39 (44.3%)	< 0.001[a]
	No	16 (18.2%)	49 (55.7%)	
Dry eyes	Yes	43 (48.9%)	27 (30.7%)	0.014[a]
	No	45 (51.1%)	61 (69.3%)	
Concentration Difficulty	Yes	68 (77.3%)	43 (48.9%)	< 0.001[a]
	No	20 (22.7%)	45 (51.1%)	
Bloating in Soft Tissues	Yes	64 (72.7%)	35 (39.8%)	< 0.001[a]
	No	24 (27.3%)	53 (60.2%)	
Fibromyalgia History in the Family	Yes	37 (42%)	0 (0%)	< 0.001[b]
	No	51 (58%)	88 (100%)	

[a]Chi-square test with n (%). [b]Fisher's exact test with n (%). [c]Student's *t*-test with mean ± standard deviation.

Table II. Comparison of protein levels between the patient and control groups.

	Patient (n = 88)	Control (n = 88)	*p*-values
Histone H3 (ng/mL)	10.51 (8.15-14.55)	9.74 (7.27-13.54)	0.184[d]
Prolidase (ng/mL)	53.97 (46.65-61.27)	3.90 (3.30-4.70)	< 0.001[d]

[d]Mann-Whitney U test with median (Quartiles: Q1 -Q3).

significantly similar between the patient and control groups (p=0.477). The average age of the patients was 43.94±8.33 and the average age of the controls was 43.18±5.56.

Sleep disturbance, fatigue, headache, morning fatigue, dry mouth, numbness in the legs, dry eyes, difficulty concentrating, feeling of bloating in soft tissues, and familial history of FM were significantly higher in the patient group than in the control group (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p=0.014, p<0.001, p<0.001, p<0.001, respectively, Table I). Histone values were not significantly different between the patient and control groups (p=0.184, Table II). Prolidase values of the patient group [53.97 (46.65-61.27) were significantly higher than those of the control group [3.90 (3.30-4.70)] (p<0.001, Table II). The boxplot of the distribution of histone values between the groups is shown in Figure 1A and the boxplot of the distribution of prolidase values is shown in Figure 2A.

ROC analysis was performed to determine whether histone and prolidase values were significant parameters in the prediction of disease. According to the ROC analysis results, histone was statistically insignificant in the prediction of disease [p=0.184, AUC: 0.558 (0.473-0.643)], (Figure 1B). Prolidase was statistically significant in predicting disease [p<0.001, AUC: 1 (1-1)], (Figure 2B). The ROC analysis results, sensitivity, selectivity, and positive/negative predictive values are presented in Table III. The prolidase's discriminatory power (ROC area under the curve) was excellent. The best cut-off point for prolidase was 15.43, and the sensitivity and specificity values for patient/control discrimination for this cut-off were 100% (100-100) with confidence intervals.

No significant correlation was found between histone and prolidase values (p=0.171). No significant correlation was found between histone and prolidase values for the control group only

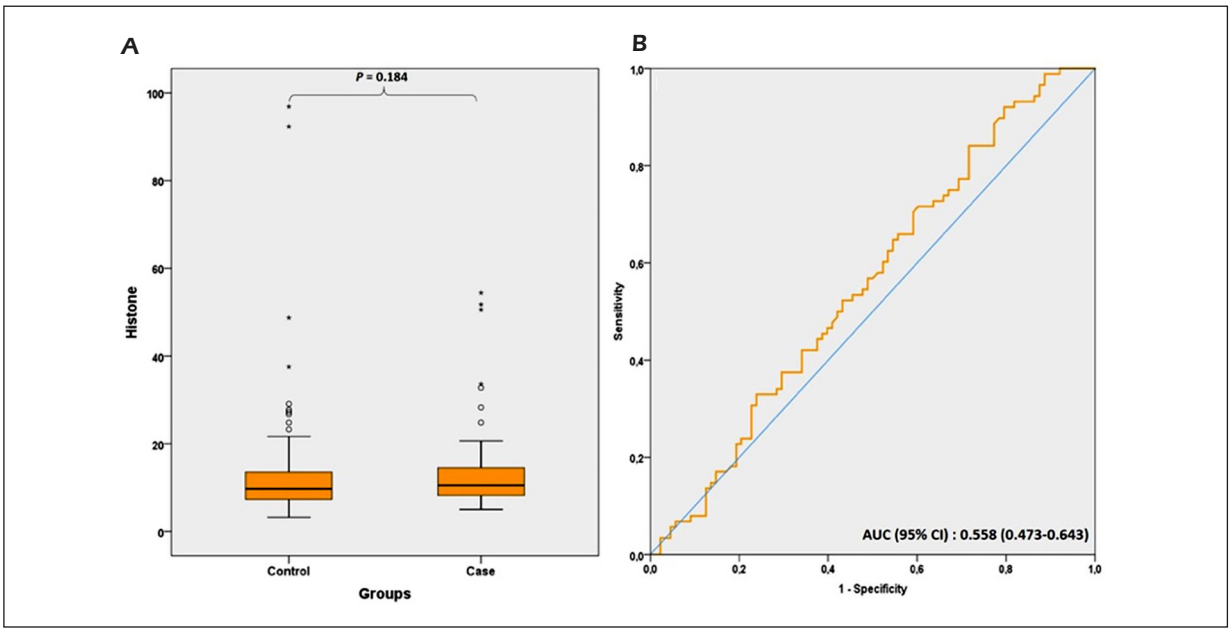

Figure 1. A, Boxplot showing the histone values distribution between the patient and control groups. **B,** The area under the ROC curve for patient/control discrimination by histone H3 values.

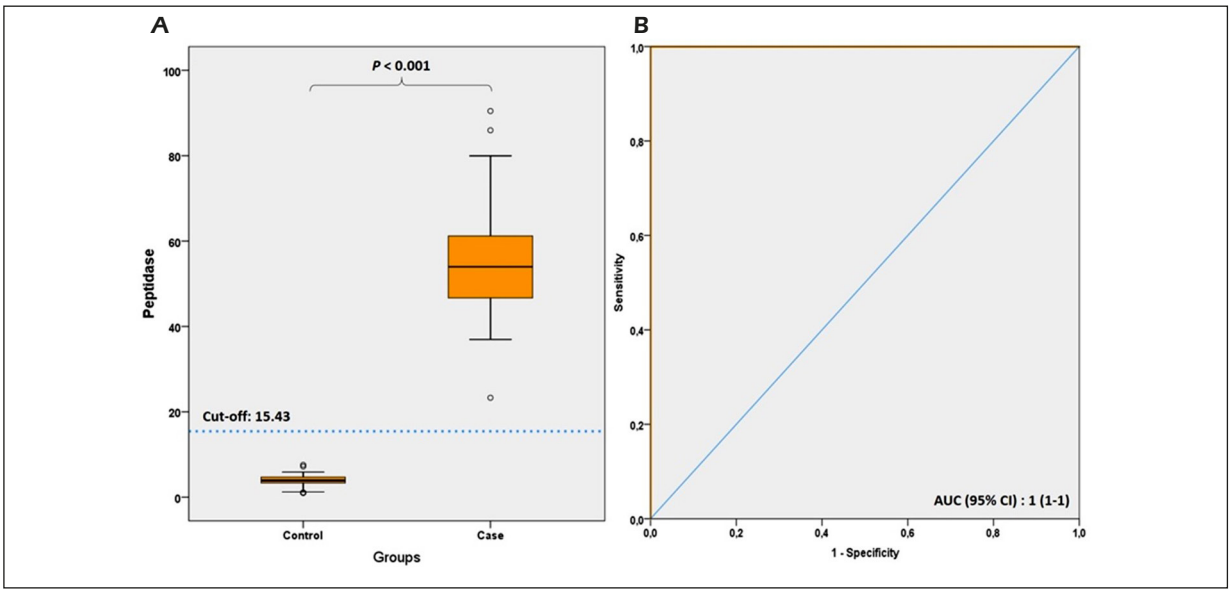

Figure 2. A, Boxplot showing prolidase values distribution between the patient and control groups. **B,** The area under the ROC curve for patient/control discrimination by prolidase values.

(p=0.963). No significant correlation was found between histone and prolidase values for the patient group only (p=0.446).

Statistical findings regarding the comparison of histone H3 and prolidase values between the research groups according to FM syndromes are given in Table IV.

Discussion

Prolidase is a member of the matrix metalloproteinase family. It is also a cytosolic exopeptidase that cleaves the C-terminals of imidodipeptides containing proline and hydroxyproline. Prolidase enzyme plays a significant role in inflammation, collagen metabolism, and cell growth. In our study, the prolidase levels of the patients were higher than those of the controls. According to the ROC analysis, prolidase was statistically significant in the prediction of disease.

Only one study[14] was encountered in the literature on FM and prolidase. The authors stated that prolidase can be used as a biomarker, and our study supports their conclusion. Eser et al[31] investigated serum prolidase enzyme activity and familial Mediterranean fever (FMF) and they found that patients with FMF had higher serum prolidase enzyme activity than the healthy control group.

Kucukdurmaz et al[15] evaluated serum prolidase activity in men with prostate cancer and benign prostatic hyperplasia (BPH). They found serum prolidase activity was similar in patients with BPH and prostate cancer and the activity of serum

Table III. Receiver operating characteristic (ROC) analysis results, sensitivity, specificity, and positive/negative predictive values for the success of prolidase and histone values in disease prediction.

	Prolidase (95% CI)	Histone H3 (95% CI)
AUC (95% CI)	1 (1-1)	0.558 (0.473-0.643)
p-values	< 0.001	0.184
Cut-off	15.43	-
Sensitivity	100% (100-100)	-
Specificity	100% (100-100)	-
PPV	100% (94.8-100)	-
NPV	100% (94.8-100)	-

AUC: Area Under the ROC Curve, CI: Confidence Interval, PPV: Positive Predictive Values, NPV: Negative Predictive Values

Table IV. Relationship between diagnostic parameters and protein levels.

Fibromyalgia syndrome		Groups	Histone H3 median (Q1-Q3)	p	Prolidase median (Q1-Q3)	p
Sleeping disorder	Yes	Controls (n = 36)	9.86 (7.28-13.07)	0.357[d]	4.29 (3.51-4.90)	< 0.001[d]
		Patients (n = 69)	10.61 (8.19-14.46)		52.98 (46.08-60.73)	
	No	Controls (n = 52)	9.25 (7.27-14.45)	0.363[d]	3.68 ± 1.34	< 0.001[c]
		Patients (n = 19)	9.67 (8.08-15.33)		59.45 ± 10.51	
Fatigue	Yes	Controls (n = 66)	9.74 (7.31-13.26)	0.134[d]	3.85 (3.33-4.83)	< 0.001[d]
		Patients (n = 84)	10.51 (8.15-14.55)		54.30 (46.74-61.37)	
	No	Controls (n = 22)	10.23 (6.41-17.86)	0.811[d]	4.10 (2.94-4.52)	< 0.001[d]
		Patients (n = 4)	11.24 (7.13-27.98)		47.89 (44.97-53.01)	
Headache	Yes	Controls (n = 46)	9.59 (8.14-11.79)	0.092[d]	3.76 (3.16-4.75)	< 0.001[d]
		Patients (n = 81)	10.61 (8.53-14.78)		53.50 (46.62-60.99)	
	No	Controls (n = 42)	10.36 (6.95-15.96)	0.605[d]	4.18 (3.38-4.42)	< 0.001[d]
		Patients (n = 7)	8.76 (6.55-13.99)		60.95 (51.70-65.41)	
Morning Fatigue	Yes	Controls (n = 47)	10.07 (7.96-13.41)	0.473[d]	4.12 ± 1.27	< 0.001[c]
		Patients (n = 83)	10.68 (8.36-15)		54.97 ± 10.11	
	No	Controls (n = 41)	8.89 (6.35-15.53)	0.811[d]	3.76 (2.82-4.37)	< 0.001[d]
		Patients (n = 5)	9.67 (6.08-10.08)		48.24 (46.38-73.42)	
Dry Mouth	Yes	Controls (n = 44)	10.02 (8.15-13.54)	0.430[d]	3.90 ± 1.16	< 0.001[c]
		Patients (n = 69)	10.68 (8.73-15.14)		54.43 ± 9.79	
	No	Controls (n = 44)	9.19 (6.64-14.04)	0.703[d]	3.91 (3.37-4.49)	< 0.001[d]
		Patients (n = 19)	9.86 (7.16-13.99)		56.49 (48.24-61.68)	
Leg Numbness	Yes	Controls (n = 39)	9.91 (7.31-15.85)	0.718[d]	3.79 ± 1.15	< 0.001[c]
		Patients (n = 72)	10.62 (8.03-14.48)		54.84 ± 9.89	
	No	Controls (n = 49)	9.67 (6.91-12.63)	0.273[d]	4.17 (3.36-4.58)	< 0.001[d]
		Patients (n = 16)	9.72 (8.71-19.10)		54.26 (45.58-64.48)	
Dry Eyes	Yes	Controls (n = 27)	10.44 (7.34-14.90)	0.267[d]	3.94 (3.45-4.87)	< 0.001[d]
		Patients (n = 43)	11.31 (9.37-15.29)		52.03 (46.03-60.83)	
	No	Controls (n = 61)	9.55 (6.95--13.46)	0.749[d]	3.88 (3.01-4.60)	< 0.001[d]
		Patients (n = 45)	9.58 (7.18-14.29)		54.79 (49.54-62.06)	
Concentration Difficulty	Yes	Controls (n = 43)	9.55 (7.27-11.85)	0.164[d]	3.75 (2.84-4.72)	< 0.001[d]
		Patients (n = 68)	10.36 (8.15-14.87)		52.94 (46.25-60.87)	
	No	Controls (n = 45)	9.91 (7.27-14.32)	0.541[d]	4.10 ± 1.18	< 0.001[c]
		Patients (n = 20)	10.66 (8.10-14.42)		58.17 ± 9.46	
Bloating in Soft Tissues	Yes	Controls (n = 35)	9.27 (6.86-11.33)	0.029[d]	3.48 ± 1.09	< 0.001[c]
		Patients (n = 64)	10.93 (8.15-14.52)		54.19 ± 9.78	
	No	Controls (n = 53)	10.63 (7.33-14.83)	0.826[d]	4.29 (3.37-4.93)	< 0.001[d]
		Patients (n = 24)	9.63 (7.56-15.13)		55.01 (48.54-61.88)	
Fibromyalgia history in the lamily	Yes	Controls (n = 0)	-	-	-	-
		Patients (n = 37)	10.61 (7.40-15.16)		54.14 (46.62-61.55)	
	No	Controls (n = 51)	9.74 (7.27-13.54)	0.275[d]	3.90 (3.30-4.70)	< 0.001[d]
		Patients (n = 88)	10.03 (8.76-14.40)		53.08 (46.71-61.09)	

[c]Student's *t*-test with mean± standard deviation. [d]Mann-Whitney U test with median (Quartiles: Q1 -Q3).

prolidase did not correlate with age in each group.

In 2020, Karacan et al[17] conducted a study on serum prolidase activity in children with epilepsy. Their study showed that epileptic patients receiving antiepileptic treatment had increased serum prolidase enzyme activity. They claimed that serum prolidase enzyme activity may be considered a significant biomarker for subclinical vascular damage in children with epilepsy on certain antiepileptic drugs.

Aktürk et al[18] investigated the relationship between serum prolidase activity and coronary

artery ectasia. They examined a patient group and a healthy control group, and they found that serum prolidase activity was significantly higher in the patient group.

Bhatnager et al[19] conducted a study on serum prolidase levels and polycystic ovary syndrome (PCOS). They examined 170 patients and 160 healthy controls. They found that prolidase levels were much higher in the PCOS group than in the control group. They also found that prolidase levels increased with the number of cysts in the ovaries.

Histone H3 plays a central role in the neutrophil release of nuclear chromatin and is also known as a NET. NETs have been shown to have detrimental effects on the host and were proposed to promote tumor progression. In our study, there was no difference between the patient group and the control group in the levels of histone H3 protein. According to the ROC analysis, histone H3 protein was statistically insignificant in the prediction of disease.

In contrast, Tian et al[46] investigated histone H3 concentrations in septic shock patients. They measured citrullinated histone H3 protein levels in serum samples of 160 patients and healthy volunteers. They found that histone H3 levels were highly increased in people with septic shock. They concluded that the concentration of histone H3 correlates with the severity of the disease and prognosis. Mauracher et al[34] conducted a study on histone H3 and the risk of venous thromboembolism (VTE) in cancer patients. Their data showed that patients with increased histone H3 levels faced a higher cumulative incidence of VTE than patients with levels below this cut-off.

Thålin et al[47] investigated plasma histone H3 levels and examined their relationship with cancer. They found an important increase in histone H3 in patients with cancer compared with healthy people. They claimed that histone H3 could be used as a biomarker.

Kuczia et al[48] investigated asthma patients and histone H3 levels as a marker. They stated that asthma was characterized by increased circulating histone H3 levels. Although more research is needed, they thought that histone H3 could be used as a biomarker for diagnosing this disease.

Conclusions

The serum levels of prolidase were elevated in patients with FM compared with the control group. However, histone H3 levels did not differ between the patient and control groups. According to the ROC analysis, prolidase was statistically significant in the prediction of disease, while histone H3 was not. Although more studies are required, it is thought that prolidase can be used as a biomarker in the diagnosis of FM.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

No funding was received.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

A. Ozturk developed the theory, analyzed the data in the conclusion, and wrote the discussion section. T. Agbektas, A. Tas, M.A. Gul, and Y. Silig evaluated the findings, reviewed, and made necessary adjustments. All authors have seen and approved the final version of the article. T. Agbektas and A. Tas prepared the necessary documents for ethics committee approval. T. Agbektas and A. Karadag collected and stored the patient samples used in the study. A. Ozturk, M.A. Gul, T. Agbektas, and A. Tas carried out the ELISA studies

Ethics Approval

An application was made to the Sivas Cumhuriyet University Clinical Research Ethics Committee (Sivas, Turkey), and the necessary ethics committee approval was obtained (Dated: 13.01.2022; Approval Number: 2022-01/34). The study was carried out in compliance with the ethical principles of the Declaration of Helsinki. The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Informed Consent

Written informed consent was obtained from all participants in the study.

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