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ORIGINAL ARTICLE

Serum Calcitonin Gene-Related Peptide and Receptor Protein Levels in Patients With Fibromyalgia Syndrome: A Cross-Sectional Study

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ABSTRACT

Objectives: This study aims to compare the serum calcitonin gene-related peptide (CGRP) and CGRP receptor protein levels between patients with fibromyalgia syndrome (FM) and healthy control subjects.

Patients and methods: The study included 88 patients (7 males, 81 females; mean age 44.5±9.1 years; range, 20 to 72 years) newly-diagnosed with FM according to the 2010 American College of Rheumatology criteria and 88 healthy volunteers (6 males, 82 females; mean age 43.0±6.1 years; range, 20 to 57 years). Venous blood samples were collected from both groups for the measurement of the levels of serum CGRP and CGRP receptor proteins (receptor component protein [RCP], receptor activity modifying protein 1 [RAMP 1] and calcitonin receptor-like receptor [CLR]).

Results: A comparison of the serum CGRP, CLR and RCP levels of the FM and control groups revealed a statistically significant difference (p=0.001, p=0.005, p=0.001, respectively). The difference between the groups in respect of the serum RAMP 1 levels was not statistically significant (p=0.107).

Conclusion: The serum CGRP, CLR and RCP levels were found to be higher in the FM patients, but no difference was determined between the FM patients and the healthy control group in respect of the RAMP 1 level. These results can be of guidance for further clinical studies of the etiopathogenesis and treatment of FM.

Keywords: Calcitonin gene-related peptide, fibromyalgia, pain, receptor proteins.

Fibromyalgia syndrome (FM) is a clinical condition with many symptoms such as diffuse pain, fatigue, sleep disturbance and cognitive dysfunction.[1] Genetic, environmental factors, immunological factors, peripheral and central mechanisms are thought to play a role in the pathogenesis of FM.[2] In previous clinical studies, it has been suggested that dysfunction in pain processing mechanisms such as peripheral and central sensitization may play a role in FM pathogenesis, and the allodynia and hyperalgesia seen in FM patients are thought to be due to this dysfunction in central mechanisms.[3,4] However, central sensitization, which is thought to play a role in FM pathogenesis, is responsible for the increase in excitatory neurotransmitters such as calcitonin gene-related peptide (CGRP), glutamate and substance P, or a decrease in inhibitory neurotransmitters such as serotonin and norepinephrine.[5-7]

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Calcitonin gene-related peptide is a 37 amino acid peptide primarily localized in the A delta and C nerve fibres.[8] CGRP is one of the excitatory neurotransmitters involved in the transmission of pain and central sensitization. Together with CGRP, CGRP receptor plays an important role in peripheral and central sensitization.[9] A functional CGRP receptor requires three components; the calcitonin receptor-like receptor (CLR), which consists of seven transmembrane domains, the receptor activity modifying protein 1 (RAMP 1), which grants specificity for binding with CGRP, and receptor component protein (RCP), which appears to be critical for signal transduction by promoting effective coupling to the Gs protein.[9]

There are studies in the literature evaluating the relationship between CGRP and pain in different diseases.[10] However, although there is chronic pain in FM, to the best of our knowledge, there is no study in the literature that has evaluated serum CGRP levels in FM. However, evaluation of the serum level of CGRP alone may not be sufficient to understand the role of CGRP in the pathogenesis of FM. Therefore, evaluation of CGRP and the CGRP receptor protein system is important for better understanding of the role of CGRP in FM. In this study, we aimed to compare the serum CGRP and CGRP receptor protein levels between patients with FM and healthy control subjects.

PATIENTS AND METHODS

This cross-sectional study was conducted in the Physical Medicine and Rehabilitation Clinic of Sivas Cumhuriyet University Medical Faculty between June 2018 and June 2019. The study included 88 patients (7 males, 81 females; mean age 44.5±9.1 years; range, 20 to 72 years) who were newly-diagnosed with FM according to the 2010 American College of Rheumatology[11] criteria and 88 healthy control subjects (6 males, 82 females; mean age 43.0±6.1 years; range, 20 to 57 years). FM patients with malignancy, rheumatic disease (osteoarthritis, Behçet's disease, rheumatoid arthritis, etc.) and those with a known history of systemic disease (hypertension, diabetes mellitus, neurological or psychiatric disease, etc.) were excluded. The control group was formed of subjects with no known disease and no medication use, recruited from hospital personnel and the relatives of patients. The sample size of study was calculated with prevalence calculation when the mean of population and standard deviation values were not known. The study protocol was approved by the Cumhuriyet University Clinical Research Ethics Committee (approval #: 2018-03/25, dated: 26.03.2018). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The sex and age of the participants were recorded. Venous blood was collected from the patient and control groups. Venous blood samples were taken at 08:00-10:00 after an eight-hour fast. The samples in anticoagulant tubes were centrifuged at 3,000 rpm for five minutes at +4°C, then the separated serum was stored at -30°C to measure serum CGRP, CLR, RAMP 1 and RCP levels. Human CGRP, CLR, RAMP 1 and RCP levels were measured with enzyme-linked immunosorbent assay kits (Sunredbio, catalogue nos: 201-12-1062, 201-12-7854, SRB-T-85538, 201-12-4742, respectively) according to the manufacturer's instructions (Sunred Biological Technology Co., Ltd, Shanghai, China).

Statistical analysis

Data obtained in the study were analyzed statistically using the IBM SPSS Statistics version 22.0 software (IBM Corp., Armonk, NY, USA). Conformity of the data to normal distribution was analyzed using the Kolmogorov-Smirnov test. A Mann-Whitney U test was applied when the parametric test assumptions could not be met. Categorical data were compared using the chi-square test. Data were expressed as number and percentage or as mean/median ± standard deviation values. A value of $p<0.05$ was considered statistically significant. Considering the 3.6% prevalence rate of FM in Turkey,[12] and the total population of the province, 88 patients were included in the study within a 95% confidence interval. The power of the study was calculated post-hoc and found to be 90.14%.

Table 1. Serum calcitonin gene-related peptide, calcitonin receptor-like receptor, receptor component protein and receptor activity modifying protein 1 levels of study groups

	FM group (n=88)		Control group (n=88)		
	Median	IQR	Median	IQR	*p*
CGRP (ng/mL)	22.65	1.55 to 104.95	3.06	0.01 to 22.25	0.001*
CLR (pg/mL)	3.96	2.75 to 7.36	3.07	2.24 to 4.71	0.005*
RCP (pg/mL)	322.95	185.96 to 538.99	2.01	0.01 to 123.88	0.001*
RAMP 1 (pg/mL)	612.58	456.91 to 912.32	656.20	120.1 to 896.78	0.107

FM: Fibromyalgia syndrome; CGRP: Calcitonin gene-related peptide; CLR: Calcitonin receptor-like receptor; RCP: Receptor component protein; RAMP 1: Receptor activity modifying protein 1; * p value <0.05; Mann-Whitney U test was used.

RESULTS

No statistically significant difference was determined between the patient and control groups in terms of age and sex (p>0.05). When the CGRP levels of the FM patients and the control group were compared, the serum CGRP level was statistically significantly higher in the FM group than in the control group (p=0.001). In the comparisons of the serum CLR, RCP and RAMP 1 levels, the CLR and RCP levels were statistically significantly higher in the FM group than in the control group (p=0.005, p=0.001, respectively). No statistically significant difference was determined between the groups in respect of serum RAMP 1 levels (p=0.107) (Table 1).

DISCUSSION

In this study, the serum CGRP and CGRP receptor components, CLR, RCP and RAMP 1 levels were evaluated in patients with FM, and the serum CGRP levels were found to be higher in patients with FM than in healthy individuals. In addition, CGRP receptor proteins CLR and RCP levels were found to be higher in patients with FM, whereas the RAMP 1 level was not different from that of healthy individual.

Calcitonin gene-related peptide modulates motor, sensory and integration systems in the central nervous system, and provides pain awareness and has an excitatory effect by increasing the release of substance P. Peripherally, however, it regulates synaptic transmission by inhibiting the expression of acetylcholine esterase at the neuromuscular junction.[13]

Since CGRP is a neurotransmitter responsible for the development of peripheral and central sensitization, CGRP levels have been evaluated in many clinical trials particularly in those related to migraine.[10] CGRP levels have been reported to be high particularly in studies conducted on the musculoskeletal system and rheumatic diseases. Onuoha and Alpar[14] showed that serum CGRP level was higher in patients with muscle injury compared to the control group. In another study, Alpar et al.[15] showed high serum CGRP levels in patients with neck and shoulder pain. In a different study, Dong et al.[16] determined that the serum CGRP level was higher in patients with knee osteoarthritis than in healthy control subjects. In a review evaluating the relationship between CGRP and arthritis, CGRP levels were reported to be higher in patients with arthritis.[17] Birklein et al.[18] showed that the serum CGRP level was higher in patients with complex regional pain syndrome compared to a control group. In different clinical studies, it has been shown that CGRP levels are high in patients with neuropathic pain.[19] In a previous study, CGRP levels in cerebrospinal fluid were measured in patients with FM, but no comparisons were made because there was no healthy control group.[20] In the current study, the serum CGRP level was high in patients with FM, as in other musculoskeletal diseases.

Although there are many receptor components of CGRP, the main receptor functioning for CGRP is accepted as CLR/RAMP 1.[21] However, to be able to see the optimal function of CLR/RAMP 1 receptor, RCP is necessary.[22] While CLR is expressed widely throughout the body, specific expression of RAMPs to tissue is known.[23,24] RCP is known to be expressed together with CGRP[25] and previous clinical

studies have shown that CGRP receptor level is increased in migraine, and inflammatory and neuropathic pain. Again, different studies have shown that CGRP receptors are high in synovial examinations of patients with osteoarthritis.[26-29] To the best of our knowledge, there is no study in the literature that has evaluated serum CGRP receptor levels in FM. However, the results of the current study of FM patients, similar to those of previous studies of different disease groups, found high levels of CLR and RCP, which are CGRP receptor components. Unlike previous studies, the serum RAMP 1 level of the patients in the current study was not found to be different from that of the control group. The reason for this different result was thought to be that RAMP 1, which is a CGRP receptor, is expressed specifically to tissue.

Limitations of this study included the lack of objective pain level measurement and lack of correlation between pain level and CGRP level.

In conclusion, serum CGRP level and CLR and RCP levels, which are components of CGRP receptor, were found to be higher in patients with FM than in healthy subjects. However, the level of RAMP 1, which is a CGRP receptor component in FM patients, was no different from that of healthy individuals. The results of this study may contribute to a better understanding of the pathogenesis of FM, and can serve as a guide for future clinical studies investigating the pathogenesis and treatment of FM. Nonetheless, there is a need for further clinical studies to evaluate the level of serum CGRP and CGRP receptor proteins in FM.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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