Calcitonin Gene-Related Peptide, Vascular Endothelial Growth Factor, and Clinical Manifestations in Women With Fibromyalgia

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Background: Fibromyalgia is a complex illness to diagnose and treat, which significantly impairs patients' quality of life.

Objectives: The study aims were to compare levels of calcitonin gene-related peptide and vascular endothelial growth factor between patients with fibromyalgia and healthy controls and to examine their relationship with the main clinical manifestations of fibromyalgia.

Methods: This case–control study included 42 women diagnosed with fibromyalgia and 22 healthy women. Serum calcitonin gene-related peptide and vascular endothelial growth factor levels were spectrophotometrically analyzed by enzyme-linked immunosorbent assay. Clinical manifestations were assessed by means of self-administered questionnaires, including functional capacity in daily living activities, musculoskeletal pain, fatigue, anxiety, and sleep quality. The predictive value of these parameters in fibromyalgia was determined by receiver operating characteristic curve analysis.

Results: Serum calcitonin gene-related peptide levels significantly increased in the fibromyalgia group in comparison to the control group. However, there were no significant differences in vascular endothelial growth factor levels between patients and controls. No significant correlations were found between calcitonin gene-related peptide and vascular endothelial growth factor and the symptoms analyzed.

Discussion: Serum calcitonin gene-related peptide levels were dysregulated in women with fibromyalgia and may be a reliable parameter to help diagnose this complex syndrome.

Key Words: calcitonin gene-related peptide • fibromyalgia • pain • vascular endothelial growth factor A

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Fibromyalgia (FM) is a chronic syndrome characterized by generalized muscle pain, fatigue, psychological distress, cognitive dysfunction, and sleep disturbances, with significantly impaired function and quality of life. FM is the third most frequent musculoskeletal condition, and its prevalence increases with age (Sarzi-Puttini et al., 2020). Research shows values of FM prevalence in the general population between 0.2% and 6.6% (Marques et al., 2017).

The physiopathology of FM is not fully clarified, complicating the diagnosis and treatment of this syndrome. The most widely used diagnostic criteria are those of the American College of Rheumatology, which were updated in 2016 (Wolfe et al., 2016). In the absence of reliable biomarkers that help the diagnosis of FM, patients must meet a certain number of set criteria of disease signs and symptoms for the diagnosis to be made. However, although diagnosis has improved with the evolution of more accurate diagnostic criteria, many clinicians still fail to recognize the syndrome (Sarzi-Puttini et al., 2020).

The main hypotheses about the physiopathology of FM include alterations in multiple ascending and descending central nervous system pathways, as well as peripheral pathways, leading to heightened pain sensitivity (Chinn et al., 2016). Alterations of the peripheral nervous system in FM involve an antidromal axonal reflex of C-fibers in response to a stimulus, which activate a wide variety of processes. These processes include release of neuropeptides such as calcitonin gene-related peptide (CGRP), glutamate, and substance P; vasodilation; vascular permeability;

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and activation of immune cells that produce serotonin, tumor necrosis factor-alpha, and cytokines (Chinn et al., 2016).

CGRP is a potent peptide vasodilator and also plays a role in the transmission of pain (Edvinsson, 2019), being involved in chronic pain states (Walsh & McWilliams, 2019). It is produced in both peripheral and central neurons (Goadsby et al., 2017).

Vascular endothelial growth factor (VEGF) is a well-known endothelial cell mitogen, associated with angiogenesis, and vascular growth and permeability factor (Namiecińska et al., 2005). Recently, a role has been proposed for VEGF in the modulation of both nociception and the onset of chronic pain (Hulse, 2017).

To our knowledge, there is only one study available that examines serum CGRP levels in FM patients (Koruku et al., 2020), as well as three studies that evaluate VEGF levels in these patients, with contradictory results (Blanco et al., 2010; Karadağ et al., 2019; Kim et al., 2010). The objective of this study was to compare serum CGRP and VEGF levels between patients with FM and controls, as well as to investigate their relationship with the main clinical manifestations of FM. In addition, we examined the ability of these molecules to differentiate individuals with FM from healthy subjects.

METHODS

Design

This case–control study was carried out in accordance with the Declaration of Helsinki of the World Medical Association. The study was approved by the Ethics Committee of the University of Granada (Spain) (approval number: 1718-N-18).

Sample Size

Sample size was determined using Ene 3.0 software (GlaxoSmithKline, Rockville, MA, USA). To achieve a power (1 – β error) of .08, taking into account a significance level (α error) of .05 and based on the results of CGRP-like activity levels in chronic pain patients (Lindh et al., 1999), it is necessary to include at least 19 subjects per experimental group in the study.

Participants

Forty-two women with FM and 22 healthy women were enrolled. Only women were selected based on FM prevalence data by gender, which is higher in women than in men (Collado et al., 2014; Mas et al., 2008). Women with FM were contacted via AGRAFIM (Association of Fibromyalgia of Granada, Spain) and AFIXA (Association of Fibromyalgia of Jaén, Spain); healthy women were recruited from friends and relatives of the patients, from friends and colleagues of the healthy subjects, and from the Faculty of Health Sciences of the University of Granada (Spain) in September 2019. We selected participants based on the inclusion and exclusion criteria proposed and according to the demographic and clinical data they provided in their first visit to our laboratories of the Faculty of Health Sciences of the universities of Granada and of Jaén (Spain) in October 2019. Each participant provided her demographic and clinical data by means of an interview and by filling out a questionnaire.

All the participants were over 18 years old. The patients had been previously diagnosed with FM by a professional rheumatologist of the Public Health System of Andalucía (Spain) and met the 1990 American College of Rheumatology criteria for FM. The exclusion criteria, both for patients and for controls, were presence of other chronic disease (diabetes mellitus, hypertension, or cancer); Grade II obesity (body mass index [BMI] of $\geq$35 kg/m^2); presence of cardiac, renal, or hepatic insufficiency; severe physical disability; psychiatric illness; previous history of surgery; pregnancy or lactation; and treatment with vasoactive drugs, anticoagulants, corticosteroids, estrogens, or agonist/antagonist opioid receptors (morphine, tramadol, oxycodone, naltrexone, etc). All participants provided written informed consent and did not receive financial incentive.

Questionnaires

In a second visit to our laboratories in December 2019, the participants completed several questionnaires, and blood samples were taken. All the questionnaires were completed by FM patients and by controls, with the exception of the Revised Fibromyalgia Impact Questionnaire (FIQ-R) that was completed only by the patients. The functional capacity in daily living activities was assessed by the Spanish version of the FIQ-R, which has an internal consistency (Cronbach's alpha) of .91 (Salgueiro et al., 2013). The questionnaire consists of 21 items, and the total score ranges from 0 to 100. Self-reported musculoskeletal pain was measured according to a visual analogue scale (VAS), whose score ranges from 0 to 100. This scale has shown high sensibility and specificity in the FM population (Marques et al., 2008). Fatigue was evaluated by the Spanish version of the Multidimensional Fatigue Inventory (MFI; Munguía-Izquierdo et al., 2012), which consists of 20 items and has a potential score range from 20 to 100. The Spanish version of the MFI has shown high internal consistency, with a Cronbach's alpha of .93 (Soriano-Maldonado et al., 2015). The anxiety-related symptoms were evaluated using a Spanish version of the Beck Anxiety Inventory (BAI; Sanz & Navarro, 2003), which has a Cronbach's alpha of .93 (Magán et al., 2008). The BAI consists of 21 items, and the score ranges from 0 to 63. Sleep quality was evaluated using the Spanish version of the Pittsburgh Sleep Quality Index (PSQI), which has an internal consistency (Cronbach's alpha) of .805 (Hita-Contreras et al., 2014). The questionnaire consists of 24 items, and the total score varies in a range of 0–21. For all these questionnaires, higher values reflect worse symptomatology.

Blood Collection and Measurement of Serum CGRP and VEGF Levels

Venous blood was taken from the antecubital vein into an anticoagulant-free tube. Blood was allowed to clot for 30 minutes at room temperature. The tube was then centrifuged at 3,500 rpm for 5 minutes at 4°C to obtain serum

samples. To avoid potential circadian variations in the levels of CGRP and VEGF, the blood samples were collected at the same time of day and by the same practitioner.

CGRP and VEGF levels were determined by enzyme-linked immunosorbent assay (ELISA) in serum samples following the manufacturer's recommendations (CGRP [human] ELISA kit, Reference #A05481.96 wells, Bertin Bioreagent; and human vascular endothelial cell growth factor A ELISA kit, Catalog No. E-EL-H0111, Elabscience, respectively). ELISA is a plate-based assay technique designed for detecting and quantifying soluble substances. Wells of the plate are coated with an antibody specific to the molecule to be quantified (target molecule). Samples are added to the plate wells, and the target molecule is combined with the specific antibody. For detection, the target molecule is subsequently complexed with another antibody that is linked to a reporter enzyme. Detection is accomplished by measuring the activity of the reporter enzyme via incubation with the appropriate substrate to produce a colored product. The intensity of color, which is determined by spectrophotometry, is proportional to the amount of the target molecule present in the well.

Statistical Analysis

The statistical analysis of the data was performed using the statistical package IBM SPSS Statistics 24 for Windows (SPSS, Inc., Chicago, IL). We have expressed data for continuous variables as mean and standard deviation. We performed the Kolmogorov–Smirnov test ($\alpha = .05$) and the Levenne test ($\alpha = .05$) to test normality and homoscedasticity, respectively. We tested data that followed a normal distribution and the principle of homoscedasticity of variances (age, BMI, MFI, BAI, PSQI, and VEGF) using an unpaired Student's *t*-test to compare differences between means. To establish the degree of statistical significance in data that did not follow a normal distribution or the principle of homoscedasticity (VAS and CGRP), we applied the Mann–Whitney *U* test. To assess the relationships between variables, we used Pearson's and Spearman's correlation coefficients as parametric and nonparametric measures of rank correlation, respectively. For the receiver operating characteristic (ROC) analysis, we used MedCalc statistical software to calculate area under the curve, cutoff point, positive and negative predictive values (PV+ and PV−, respectively), sensitivity, and specificity. The Youden index was used to determine the cutoff point of the variable in the ROC curve (Youden, 1950). We set the level of statistical significance at $p < .05$.

RESULTS

Demographic and Clinical Data

Sixty-nine women with FM were voluntarily enrolled to participate in this study, and 42 were selected based on the proposed inclusion and exclusion criteria. Forty healthy women were also recruited, but 18 were excluded for meeting the exclusion criteria or for not matching with FM patients in age and BMI, resulting in a final control group of 22. Table 1 shows the demographic and clinical data of the participants. Both FM and control groups were comparable in terms of age and BMI ($p > .05$). The VAS, MFI, BAI, and PSQI scores of the women with FM were significantly higher than those of the healthy women (all ps $< .001$).

Serum CGRP and VEGF Levels

Serum CGRP levels were significantly higher in women with FM than in controls ($p < .05$), whereas the serum VEGF levels

TABLE 1. Demographic and Clinical Data of Women With FM and Healthy Women

	Healthy women (*n* = 22)		Women with FM (*n* = 42)			
Variable	***M***	***SD***	***M***	***SD***	**Kolmogorov–Smirnov test**	**Statistical test[a]**
Age (years)	54.77	7.78	56	6.73	$Z = 0.515$ ($p = .954$)	$t = 0.656$ ($p = .514$)
BMI (kg/m^2)	26.52	4.39	27.79	6.53	$Z = 0.865$ ($p = .443$)	$t = 0.821$ ($p = .415$)
FIQ-R	—	—	73.56	13.37	$Z = 0.658$ ($p = .779$)	—
VAS	12.38	22.78	74.29	16.98	$Z = 1.743$ ($p = .005$)	$U = 43$ ($p < .001$)
MFI	40.00	16.79	81.02	10.06	$Z = 1.335$ ($p = .057$)	$t = 12.226$ ($p < .001$)
BAI	9.55	11.32	33.10	8.79	$Z = 0.930$ ($p = .352$)	$t = 9.199$ ($p < .001$)
PSQI	5.64	3.95	15.63	3.64	Z = 1.128 ($p = .157$)	$t = 10.019$ ($p < .001$)

Note. FM = fibromyalgia; *M* = mean; *SD* = standard deviation; BMI = body mass index; FIQ-R = Revised Fibromyalgia Impact Questionnaire; VAS = visual analogue scale; MFI = Multidimensional Fatigue Inventory: BAI = Beck Anxiety Inventory; PSQI = Pittsburgh Sleep Quality Index.

[a]Age, BMI, MFI, BAI, and PSQI were analyzed using an unpaired Student's *t*-test, and VAS was analyzed using the Mann–Whitney *U* test.

TABLE 2. Serum CGRP and VEGF Levels in Women With FM and Healthy Women

Variable	Healthy women (*n* = 22)		Women with FM (*n* = 42)			
	M	*SD*	*M*	*SD*	Kolmogorov–Smirnov test	Statistical test[a]
CGRP (pg/ml)	35.22	3.36	47.53	39.97	$Z = 3.179$ ($p < .001$)	$U = 314$ ($p = .036$)
VEGF (pg/ml)	386.48	260.56	321.04	259.99	$Z = 1.125$ ($p = .159$)	$t = -0.956$ ($p = .343$)

Note. FM = fibromyalgia; *M* = mean; *SD* = standard deviation; CGRP = calcitonin gene-related peptide; VEGF = vascular endothelial growth factor.

[a]CGRP was analyzed using the Mann–Whitney *U* test, and VEGF was analyzed using an unpaired Student's *t*-test.

of the FM patients did not differ from those of the healthy women (Table 2).

ROC Curve Analysis

The predictive value of serum CGRP and VEGF levels in FM was determined by ROC curve analysis. Only serum CGRP levels showed a significant area under the curve (0.660; 95% CI [0.531, 0.774], $p = .022$). Several data related to the ROC curve for CGRP levels were calculated, including the cutoff point (36.66 pg/ml), sensitivity (52.38%), specificity (81.82%), PV+ (6.6), and PV− (98.6). The predictive values indicate that the probability of reliably cataloguing a person with serum CGRP values of ≤36.66 pg/ml as healthy is 98.6% and that 6.6% of subjects who have serum CGRP values of >36.66 pg/ml may be diagnosed with FM with a sensitivity of 52.38% and a specificity of 81.82%. Figure 1 shows the ROC curve for serum CGRP levels.

Correlations Between Variables in Patients With FM

No significant correlations were found between serum levels of CGRP and VEGF and the demographic and clinical data in patients with FM (Table 3).

DISCUSSION

FM is a complex multisymptom disorder. The high prevalence of FM in the adult population, the difficulty in the diagnosis, and the absence of effective treatment involve a high health, personal, and social burden. The development of FM results from augmented pain processing, whereby the exact mechanisms are unknown. In this study, we aim to examine serum levels of CGRP and VEGF in patients with FM and in healthy

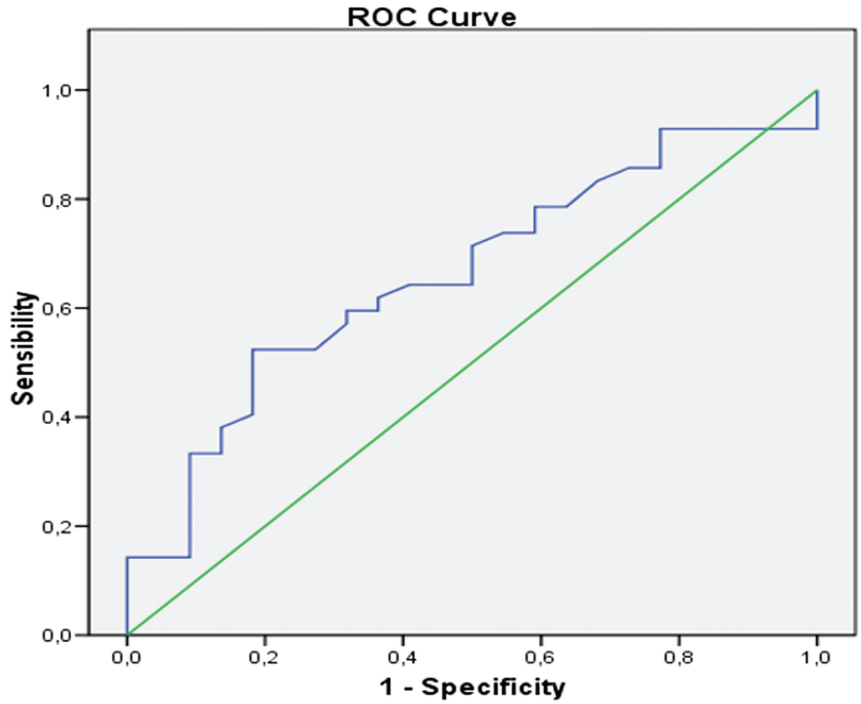

FIGURE 1. Receiver operating characteristic (ROC) curve for serum calcitonin gene-related peptide (CGRP) levels. *x*-axis = 1 minus specificity of ROC analysis; *y*-axis = sensibility of ROC analysis; clear line = reference line; dark line = ROC curve for serum CGRP levels in women with fibromyalgia. This figure is available in color online (http://www.nursingresearchonline.com).

TABLE 3. Correlations Between Variables in Patients With Fibromyalgia

Variable	CGRP	VEGF
Age	$\rho = -.226$ ($p = .151$)	$r = .219$ ($p = .164$)
BMI	$\rho = -.183$ ($p = .246$)	$r = -.035$ ($p = .824$)
FIQ-R	$\rho = -.291$ ($p = .069$)	$r = -.050$ ($p = .757$)
VAS	$\rho = .154$ ($p = .330$)	$r = .026$ ($p = .872$)
MFI	$\rho = -.115$ ($p = .467$)	$r = -.255$ ($p = .103$)
BAI	$\rho = .036$ ($p = .821$)	$r = -.174$ ($p = .271$)
PSQI	$\rho = .057$ ($p = .727$)	$r = .045$ ($p = .784$)
VEGF	$\rho = -.155$ ($p = .328$)	—

Note. r = Pearson's correlation coefficient; ρ = Spearman's correlation coefficient; CGRP = calcitonin gene-related peptide; VEGF = vascular endothelial growth factor; BMI = body mass index; FIQ-R = Revised Fibromyalgia Impact Questionnaire; VAS = visual analogue scale; MFI = Multidimensional Fatigue Inventory: BAI = Beck Anxiety Inventory; PSQI = Pittsburgh Sleep Quality Index.

controls, as well as to explore their relationship with the clinical manifestations of this syndrome.

CGRP has been involved in chronic pain states such as migraine, musculoskeletal pain including arthritis, neuropathic pain, and gastrointestinal pain (Walsh & McWilliams, 2019). Recently, Theoharides et al. (2019) suggested that future research should focus on identifying novel molecules that are involved in pain transmission such as CGRP in serum of patients with FM. At that time, there were no studies available that analyzed the serum levels of this molecule in FM patients. We have found that serum CGRP levels were higher in women with FM than in controls, coinciding with the only study that has examined serum CGRP levels in patients with FM (Korucu et al., 2020). These altered levels suggest that CGRP may play a role in the physiopathology of this complex syndrome, whose main and most disabling symptom is pain. CGRP is an excitatory neurotransmitter that facilitates pain transmission and contributes to the development and maintenance of peripheral and central sensitization and the associated enhanced pain (Iyengar et al., 2017), this being the main hypothesis regarding the physiopathology of FM.

Peripheral sensitization begins after a damage to a tissue or nerves in the periphery, leading to the release of proinflammatory mediators that can lower the activation threshold of peripheral nociceptors or activate the nociceptors, resulting in peripheral sensitization and hyperalgesia. This can cause release of excitatory transmitters such as CGRP, glutamate, and substance P from nerves, which spreads the inflammatory response. Persistent activation of the nociceptors over time results in increased production of CGRP and substance P, leading to enhanced excitability of the peripheral nerves, thus maintaining a state of peripheral sensitization with the subsequent enhanced pain. Regarding central sensitization, the increased activation of peripheral nerve terminals increases the excitability of the second-order neurons, leading to neuronal changes that increase nociceptive transmission. These changes include the activation of the CGRP receptor after releasing this neuropeptide from peripheral nerves, thereby activating a number of signaling cascades, leading to enhanced neuronal excitability, neurotransmitter release, nitric oxide production, and vasodilation (Iyengar et al., 2017). In this regard, serum levels of two CGRP receptor components (calcitonin receptor-like receptor and receptor component protein) were found to be higher in patients with FM than in controls (Korucu et al., 2020). Over time, the increased excitability of the second-order neurons results in the maintaining of a state of central sensitization, characterized by hyperalgesia and allodynia (Iyengar et al., 2017), being both symptom characteristics of patients with FM. Our results have also shown that serum CGRP levels may be a reliable parameter to diagnose FM. Serum CGRP values of >36.66 pg/ml allow distinguishing women with FM from healthy women with high sensitivity and specificity.

In addition, we assessed correlations to determine whether serum CGRP levels influence pain or other FM-related clinical features in our patients. To our knowledge, this is the first study that examines relationships between CGRP levels and clinical manifestations in FM patients. We did not find significant correlations with the symptoms analyzed in this study. These symptoms were measured using self-administered questionnaires, but the patients were guided in questionnaire completion by a specialist to aid in correct completion. Further research is needed to determine if increased CGRP levels influence pain in FM patients.

The expression of VEGF is augmented in various pain-related pathologies such as osteoarthritis (Hamilton et al., 2016) and systemic lupus erythematosus (Heshmat & El-Kerdany, 2007). VEGF can directly stimulate nociceptive sensory neurons, sensitizing nociceptors and increasing pain sensitivity after binding to its receptors, VEGFR-1 and VEGFR-2. In fact, inhibition of VEGF signaling led to reduction of pain sensitivity (Hamilton et al., 2016). In this study, we did not find significant differences in serum VEGF levels between women with FM and healthy women, similar results to those previously reported (Karadağ et al., 2019). On the contrary, lower plasma VEGF levels were found in patients with FM in comparison to the general population (Blanco et al., 2010). These conflicting results may be explained by the selection of the patients in each study. Patients with FM frequently have many comorbidities (Lichtenstein et al., 2018) that are not usually taken into account during the selection of patients and that could account for the different results in VEGF levels reported in these studies.

We also examined the relationships between serum VEGF levels and some clinical characteristics in FM patients. We failed to find significant correlations between VEGF levels and clinical manifestations, such as severity of disease, pain,

fatigue, sleep quality, or anxiety. On the contrary, though previous researchers reported a positive correlation between serum VEGF levels and the number of tender points in patients with FM (Karadağ et al., 2019), other researchers found that serum VEGF levels correlated with stiffness, reporting that VEGF levels were significantly lower in FM patients with stiffness than in patients without stiffness (Kim et al. 2010). Thus, the involvement of VEGF in the symptomatology of FM is not yet clear because it has been reported that high levels of VEGF correlated with a greater number of tender points (Karadağ et al., 2019) and that low levels of VEGF correlated with increased stiffness in FM patients (Kim et al. 2010). Our results suggest that VEGF does not seem to play a role in the physiopathology of FM. However, future studies are needed to clarify the involvement of VEGF in this complex syndrome.

The main limitation of this study is the small sample size, which makes our findings preliminary. Additional studies are needed to verify the results.

Conclusions

The higher serum levels of CGRP found in patients with FM in comparison to healthy subjects suggest that this neurotransmitter could be involved in the physiopathology of FM. The determination of serum CGRP levels may aid in the diagnosis of FM, together with the clinical data of the patient. These results are relevant for nursing science because they contribute to the knowledge about FM, which may ultimately help in the diagnosis, management, and treatment of this syndrome.

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The authors have no conflicts of interest to report.

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