FIBROMYALGIA: A SEARCH FOR MARKERS AND THEIR EVALUATION THROUGHOUT A TREATMENT

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Abstract

Fibromyalgia syndrome (FM) is a complex chronic pain disorder of unknown causation associated with debilitating fatigue, unrefreshing sleep, cognitive and affective symptoms. There is no biological markers to monitor FM progression and no permanent cure for FM. Our aim was to identify markers associated with FM and its progression and to evaluate the efficacy of a battery of treatments. The study is a treatment trial, open label and single centre, with 27 women (41 ± 2 years) diagnosed with FM using the Widespread Pain Index (WPI), the Symptom Severity (SS) Scale and the Fibromyalgia Impact Questionnaire (FIQ). Anthropometric parameters, plasma cytokines values and clinical progression were measured before and after two months of a multi-approach treatment. A significant improvement was observed after two months of treatment as shown by WPI, SS Scale and FIQ. No significant variations were observed, except for the intracellular body water parameters, in anthropometric and body composition characteristics. Food-induced histaminosis release was observed to cow´s milk, egg, fruit, wheat and oily fish. Interleukin-1β (IL-1β) and interleukin-6 (IL-6) plasma cytokine values were significantly higher in FM. A strong positive correlation was observed between the percentage of reduction of cytokine levels and the improvement of health status. We propose: i) the

existence of different subsets of FM patients; ii) the use of intracellular body water and plasma cytokine values as positive markers for FM progression; iii) that food-sensitisation could be an important mechanism for FM pathogenesis and iv) the use of a multidisciplinary approach for FM treatment.

Keywords: Fibromyalgia, body composition, food intolerance, cytokines

Introduction

FM is a common chronically painful, frequently disabling disorder of unknown origin. Patients have a low pain threshold, stiffness and tenderness, fatigue, impaired memory, and depression. Some of them also have gastrointestinal problems. Recent epidemiological studies estimate its global prevalence at 2–8% with a female/male ratio predominance (Clauw; 2014). In Spain, the prevalence of fibromyalgia is approximately 2.4%, being more frequent in rural (~4.1%) than in urban settings (~1.7%) (Mas et al., 2008).

There is no permanent cure for FM, therefore, adequate symptom control should be goal of treatment. The problem is that FM patients respond differently to treatments. This is probably due to the different pathogenic mechanisms involved in FM. The pathophysiological hallmark is a sensitized or hyperactive central nervous system that leads to an increased gain on pain and sensory processing (Clauw; 2015). In addition, other pathophysiological mechanisms such as mitochondrial dysfunction, oxidative stress, an inflammatory component (Sanchez-Dominguez et al., 2015) and neuroendocrine disturbances (Neeck, 2002) should be added. Moreover, overweight or obese has been associated with an increased risk of FM especially among those who also reported low levels of physical activity (Mork et al., 2010). For this reason, different medical treatments are used to treat FM and the recent guidelines suggest that the optimal treatment consists in a multidisciplinary approach with a combination of pharmacological and non-pharmacological treatment modalities (Rossi et al., 2015).

Another problem is that nowadays FM diagnosis is made solely on clinical basis, as no validated biological markers associated with the disease have been identified. Thus, identification of markers persistently associated with FM will help to effectively diagnose the illness, follow its progression and more importantly, to monitor the effects of therapeutic approaches.

The aim of this study is to identify markers associated with FM and its progression and to evaluate the efficacy of a battery of treatments.

Materials and Methods

Study sample and characteristics

This study is an interventional clinical trial (treatment trial) open label and single centre, that included 27 women aged 41 ± 2 years (range: 19-67 years) who were diagnosed with FM by an internist from the FM Unit of Viamed Santa Ángela de la Cruz Hospital and met the American College of Rheumatology Criteria for FM. Exclusion criteria for patients were not having other rheumatic disease and/or sever somatic or psychiatric disorders such as cancer, sever coronary disease, or schizophrenia. Other exclusion criteria were cardiac pacemaker, electrically conductive implants in the brain, pregnancy and being younger than 18 years old. All patients were assessed by the same researchers to reduce interexaminer error. All the participants were informed about the study aims and methodology. In addition, all of them signed a written informed consent to participate. The study was approved by the Ethics Committee of the University Pablo Olavide (Seville, Spain).

During the first visit to the FM Unit, FM diagnosis was made, together with all tests and studies. This procedure was carried out on two separate days, with at least 48h between each session. This was done in order to prevent fatigue and flare-ups. Then the treatment was prescribed and patients returned to the FM Unit after 66 ± 2 days. Afterwards, FM severity was assessed and all tests were performed again, following the same procedure as in the first visit.

The treatment prescribed consisted on (table 1): i) Ubiquinol (the fully reduced form of coenzyme Q_{10}; ii) Nutritional complex containing amino acids, vitamins and mineral; iii) Palmitoylethanolamide (PEA); iv) Synbiotic health supplement; v) Repetitive transcranial electromagnetic stimulation (rTMS); vi) Food exclusion and vii) Physical activity.

Concerning rTMS, stimulation sessions occurred once per week for eight consecutive weeks. Sessions were scheduled along the day and lasted 20 min. They occurred inside a Faraday cages to reduce environmental electromagnetic interference. Stimulation was delivered via a custom-built magnetic stimulator. Briefly, a flexible electroencephalography (EEG) cap with 33 stimulation coils was placed over the patient's head. A digital electronic generator fed the same oscillating current of intensity to all coils. The current amplitude was 545 μA. Each coil produces a magnetic field of approximately 43 nT at a distance of 1 cm and 0.9 nT at a distance of 4 cm. A low-frequency (8 Hz) square function was used. The relative fluctuations in voltage (noise) around the theoretical square function to be applied were approximately 3%.

Table 1. Treatment prescription details.

Treatment	Characteristics
Ubiquinol	200 mg/day single-dose oral during 60 days
Nutritional complex	Single-dose oral, during 60 days. It contains amino acids (arginine, methionine and glycine), vitamins (D3, E, C and B12) and minerals (magnesium, potassium, phosphorus, calcium and chromium)
PEA	400 mg/day single-dose oral during 60 days
Synbiotic	It contains probiotics (Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus plantarum and Lactococcus lactis) and prebiotics (inulin)
rTMS	1 session/week for 8 consecutive weeks, 20 min duration, 545 μA current amplitude, 43 nT at 1 cm, 8 Hz frequency
Food exclusion	Milk (100%), Egg (61%), fruit (37%), wheat (32%) and oily fish (26%) of patients
Physical activity	45-minutes walking/twice a week at 60% VO_{2max}

Anthropometrics measurements

Height (in centimeters) and weight (in kilograms) were measured using a mechanical patient weighing scale with a stadiometer (Seca 711, Hamburg, Germany). Total body fat (%), trunk fat (%), total body water (Kg), intracellular body water (kg) and phase angle (degrees) were measured by means of an eight-polar multifrequency bioimpedanciometer (BIA) (Promis Cardiofitness, Cadiz, Spain). Body Mass Index (BMI) was calculated by dividing patients´weight (in kilograms) by their height (in meters) squared.

Fibromyalgia diagnosis and severity

FM diagnosis and severity was assessed using the WPI, the SS Scale (Wolfe et al.; 2011) and the Spanish version of the FIQ (Rivera and Gonzalez, 2004).

Food-induced histaminosis release test

Patient´s heparinized blood was incubated with different food allergen extracts (cow´s milk, pork, chicken, beef, white fish, oily fish, egg, legumes, wheat, rice, tomato, orange, banana, apple, almond and hazelnut) for 30 min, at 37ºC. Then, specific histamine release was stopped by adding PBS/Tween at 4ºC. Samples were centrifuged for 15 minutes at 5,000 rpm using a refrigerated centrifuge. Extraction and purification of histamine from plasma was performed by dialysis (dialyzer membrane C12-24, Axflow, Madrid, Spain) and subsequent condensation with O-phthalaldehyde solution. Finally, histamine fluorometric measurement was performed at 640 nm. The threshold of histamine release was defined as the minimum concentration of food antigen to induce a 7% net histamine release.

Cytokine measurements

Whole blood cell was collected into commercially available anticoagulant-treated tubes (EDTA-treated). Then, cells are removed from plasma by centrifugation for 15 minutes at 2,000 *g* using a refrigerated centrifuge. The resulting supernatant was immediately transferred into clean polypropylene tubes and stored at -20ºC.

Cytokines were determined from plasma using Multiplex Luminex assay (Human Cytokine 10-Plex Panel, Life Technologies, Madrid, Spain) according to manufacturer instructions.

Statistical analysis

All data are presented as means ± standard error of the mean (SEM). All secondary analysis was performed using GraphPad InStat software (GraphPad, La Jolla, CA, USA). Analysis of variance (ANOVA) was performed to data. Thereafter, a paired and unpaired *t*-test was undertaken. $P<0,05$ was considered significant. Pearson Product Moment correlation was also calculated.

Results

Table 2 shows that a very significant improvement was observed after two months of treatment. In fact, the three outcomes measured, widespread pain index, health status (FIQ) and current pain intensity (SS scale) were extremely significant ($p< 0.001$) after two months of intervention. However, due to the complexity of the treatment and the study design, it was not possible to really understand the meaning and the contribution of the different components of the treatment.

Table 2. Values of FM diagnostic tests before and after the treatment.

FM diagnostic tests	Before treatment	After treatment
WPI	9.7 ± 0.6	5.7 ± 0.6*
SS Scale	7.6 ± 0.3	4.5 ± 0.4*
FIQ	61.6 ± 2.7	39.6 ± 4.0*

*$P< 0.001$ before treatment *vs* after treatment.

When looking at anthropometric and body composition characteristics of the study sample (table 3), before and after treatment, no significant variations were observed, except for the intracellular body water parameters ($p< 0.05$). In addition, it was observed that: i) patients´ BMI index was within normal range; ii) all patients had a severe intracellular dehydration and iii) patients didn´t have cellular damage (measured by phase angle).

Table 3. Anthropometrics characterizes before and after the treatment.

Anthropometrics characterizes	Before treatment	After treatment
BMI	24.3 ± 1.1	23.2 ± 1.0
Total body fat (%)	34.4 ± 1.8	33.6 ± 1.6
Trunk fat (%)	34.6 ± 1.7	33.4 ± 1.6
Total body water (Kg)	30.3 ± 0.5	29.5 ± 0.5
Intracellular body water (Kg)	15.1 ± 0.3	14.1 ± 0.3*
Phase angle (degrees)	4.3 ± 0.1	4.2 ± 0.3

*P< 0.05 before treatment *vs* after treatment.

Food-induced histaminosis release tests showed that patients were above threshold values for cow´s milk (100%), egg (61%), fruit (orange, banana and apple, 37%), wheat (32%) and oily fish (26%). Due to the fact that food exclusion was included within the whole treatment design, it was difficult to evaluate the real effect of food exclusion in patients´ improvement.

Plasma cytokine levels (IL-1β, IL-6 and TNF-α) was performed to determine the pathophysiological role of pro-inflammatory cytokines in FM treatment and progression. As it is observed in table 4, patients with FM had IL-1β and IL-6 plasma values higher than those reported in the literature for control subjects (Togo et al., 2009). Moreover, the treatment significantly (p< 0.001) decreased both cytokines plasma values. When comparing the improvement of health status (FIQ) with interleukins percentage of reduction, the Pearson's correlation between the two variables showed a positive Pearson Product Moment correlation of 0.59 between FIQ and IL-1β reduction and of 0,62 between FIQ and IL-6 reduction (data not shown). Both data indicated a fairly strong relationship between health status improvement and cytokines plasma values.

Table 4. Plasma cytokines values before and after treatment.

Plasma cytokines levels (pg/ml)	Before treatment	After treatment
IL-1β	24.2 ± 1.4	11.5 ± 0.3*
Il-6	5.4 ± 0.4	2,2 ± 0.1*
TNF-α	5.6 ± 0.4	5.3 ± 0.3

*P< 0.001 before treatment *vs* after treatment.

Discussion

Since 2010, FM is diagnosed according to the criteria stablished by The American College of Rheumatology (Wolfe et al.; 2011), while patients generally have a normal laboratory profile (Yunus, 2002). In addition, the pathophysiological mechanisms of FM are not well understood and several mechanisms have been proposed. However, the existence of different subtypes of FM patients with different etiology, clinical characteristics and biological markers could help to understand the situation and therefore, to

design more appropriate treatments for distinct subgroups. The existence of subpopulations of FM patients was already proposed by Caro (1989).

In this regard, looking to table 3, we observed that our patients´ group has a BMI index within normality. However, it has been described that women with a BMI greater than or equal to 25 had a 60% to 70% greater risk of developing fibromyalgia, when compared with their thinner counterparts. In addition, for a women population, aged 40 years and over, the co-ocurrence of FM with having chronic diseases, such as obesity, were strongly related (Rusu et al., 2015). In addition, our group of patients showed a severe intracellular dehydration. We do not know the pathophysiological meaning of this finding, however, a significant reduction of dehydration was observed after treatment and after patients´ clinical improvement. This raises the possibility of using intracellular dehydration as a marker of FM progression.

Our group of patients also has higher plasma levels of IL-1β and IL-6. These cytokines values were significantly reduced after treatment and after clinical improvement, with a strong correlation. This suggests that, within this subgroup of patients, the presence of an inflammatory response highlighted a parallel between the clinical symptoms and biological markers.

Among the factors suspected of promoting FM are food sensitivities and intestinal hyperpermeability (Werbach, 2000). The food-induced histaminosis release test showed that the majority of FM patients showed sensitisation to cow´s milk, egg, fruit, wheat and oily fish. This sensitisation could be increased in the presence of increased intestinal permeability. In this regard, central sensitisation has been proposed to contribute to FM pathogenesis (Nijs et al., 2012).

Finally, the FM patients significantly improved with the treatment, as shown by the FM diagnostics tests (table 2). The treatment was composed by seven items and we still don´t know the importance and effects of the different components of the treatment in our group. Additional research is necessary to further elucidate this aspect.

Conclusion

Our data indicate the existence of different subsets of FM patients. In addition, intracellular body water and plasma cytokine values could be used as positive markers for FM progression. Food-sensitisation could be an important mechanism for FM pathogenesis. Finally, due to the complexity of FM a multidisciplinary approach should be the goal of treatment.

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