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Short communication

Evaluation of cytokines, oxidative stress markers and brain-derived neurotrophic factor in patients with fibromyalgia – A controlled cross-sectional study

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ABSTRACT

Objectives: Previous studies measuring serum levels of biomarkers of inflammation/oxidative stress and neurotrophins levels in fibromyalgia (FM) have rendered inconsistent results. In the present study, our aim was to explore the levels of interleukins, oxidative stress markers and brain-derived neurotrophic factor (BDNF) in patients with FM in relation to depression and severity of disease.
Methods: In a prospective controlled cross-sectional study, serum concentrations of IL-6, IL-8, IL-10, TNF-α, thiobarbituric acid reactive substances (TBARS), protein carbonyl and BDNF were measured in 69 FM patients and 61 healthy controls (all women). In the FM group, the Fibromyalgia Impact Questionnaire (FIQ), the Beck Depression Inventory (BDI) and the Hamilton Depression Rating Scale (HDRS) were applied. Mann Whitney's and Spearman correlation tests were used for statistical analysis.
Results: The FM patients demonstrated a significant impact of the disease on quality of life (FIQ 70.2 ± 17.8) and most of them had depression at some level (82.6% and 87.0% as assessed by BDI and HDRS, respectively). Most biomarkers (IL-6, IL-8, TNF-α, TBARS and protein carbonyl) and BDNF did not differ significantly between patients and controls, but the IL-10 levels were higher in FM patients (adjusted $p = 0.041$). Among FM patients, there was no correlation of HDRS, FIQ, and BDI scores with any biomarker tested here.
Conclusion: We observed no significant differences in biomarkers between FM patients and controls, except for higher levels of IL-10 (an anti-inflammatory cytokine) in patients. The levels of biomarkers were not correlated with parameters of disease and depression severity.

1. Introduction

Fibromyalgia (FM) is a common disease with a complex and not completely known pathophysiology. Along with generalized pain, depression is frequently present in the disease context, which a prevalence ranging from 28.6% to 70% [1].

There are no reliable laboratory markers of disease activity or severity in FM. Studies evaluating levels of interleukins (IL), including IL-6, IL-8, IL-10, IL-4, and IL-2, as well as tumor necrosis factor (TNF-α) in FM, have rendered inconsistent results [2–6]. Higher levels of brain-derived neurotrophic factor (BDNF) was found in blood and cerebrospinal fluid (CSF) of FM patients [7–9]. There is also evidence of increased levels of biomarkers of oxidative stress (like protein carbonyl content and Thiobarbituric Acid Reactive Substances (TBARS) in FM [10,11]. However, it is possible that the interplay between biomarkers is more important than their isolated levels in certain diseases. In view of that, Kapczinski and colleagues developed a multi-biomarker score for mood disorders called Systemic Toxicity Index (STI), which differentiated patients with mania or depression from healthy control subjects [12]. The STI has not been studied in patients with FM so far.

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Our aim in this study is to investigate the serum concentrations of cytokines (IL-6, IL-8, IL-10 and TNF-α), BDNF and stress oxidative biomarkers (carbonyl and TBARS), as wells as their interconnection using the STI, in patients with FM in comparison to healthy controls. We also test the possibility of association of the biomarkers with the severity of FM and depression in these patients.

2. Patients and methods

2.1. Study design and participants

A prospective controlled cross-sectional study was conducted at the Rheumatology Departments of the Hospital das Clínicas da Universidade Federal de Pernambuco (HC/UFPE) and Hospital de Clínicas de Porto Alegre (HCPA). Subjects' recruitment and data collection occurred between Sep/2011 and Nov/2012.

The patients were consecutively selected from the outpatient clinic specialized in the care of fibromyalgia patients at the HC/UFPE. All subjects fulfilled both the American College of Rheumatology 1990 and 2010 criteria for diagnosis of FM. Patients had to be women aged 30–55 years, and were excluded if they had any of the following criteria: (1) current pregnancy or nursing; (2) presence of any concomitant autoimmune, inflammatory or infectious disease; and (3) diagnosed psychiatric illnesses, except for depression and/or anxiety.

Healthy women were recruited among the hospital's employees of the HC/UFPE and could not have any kind of chronic or acute pain. This control group was selected among the volunteers to form a group that matches the mean age of the patients. The control group had to follow the same exclusion criteria of FM patients, except that they could not have diagnosis of depression/anxiety disorders or use (current or past) of antidepressants or any other medication for psychiatric disorders. This group was asked specifically about the presence of symptoms of anxiety and depression (hopeless, sadness, sleep or appetite disturbance, cognitive difficulties, little interest in doing things, fatigue, and irritability), which should be completely absent.

Written informed consent was obtained from each subject and all experimental procedures were approved by the local ethics committee in both institutions (HC/UFPE and HCPA).

2.2. Study protocol and blood sample collection

Each FM patient was evaluated in two sequential stages during a single visit. First, an interviewer carried out the specific procedures of the study protocol, recording demographic and clinical features and treatment of the disease. After that, the same interviewer completed the Fibromyalgia Impact Questionnaire (FIQ) and Beck Depression Inventory (BDI) questionnaires, which are validated for application to Brazilian patients [13,14], and a translated version of the Hamilton Depression Rating Scale (HDRS) [15]. For the control group, an interview and a simple clinical exam were done to evaluate the inclusion and exclusion criteria.

After these procedures, for each subject in the study, blood samples were collected (10 mL) into an anticoagulant-free vacuum tube. All blood samples were collected in the afternoon, between 01:00 p.m. and 02:00 p.m. (fasting was not required); the blood was centrifuged (4000 rpm for 10 min) for separation of serum. The specimens were immediately aliquoted, frozen and stored at −80 °C. All samples were tested at the Molecular Psychiatry Laboratory at HCPA in a single run to reduce interassay variability. The serum concentrations of cytokines (IL-6, IL-8, IL-10, and TNF-α) were measured using Cytometric Bead Array (CBA), BNDF levels were determined using ELISA, carbonyl and TBARS were measured using specific methods (for further details, see the Supplementary Text).

2.3. Statistical analysis

Statistical analyses were made using the program SPSS version 20. The results are expressed as numbers and percentages for categorical variables, while continuous variables were presented as means ± standard deviation (SD) and/or median and interquartile range (IQR). The unpaired Student's *t* test (variables with normal distribution) or Mann-Whitney test (non-normal continuous variables) were used to evaluate the statistical significance of differences between groups. For correlations between biomarkers and clinical parameters, Spearman correlation coefficient (r_s) was used given the non-normal distribution of all biomarkers tested. Confidence intervals for correlation coefficients were calculated using the bootstrapping method with 1000 iterations. The possibility of non-linear associations was tested displaying scatter plot graphics and comparing the curves that best fitted the data using the R-square statistic. An analysis of principal components was planned to extract the STI from the shared variance of the serum biomarkers if a consistent network of correlations [12], including most or all biomarkers, was found. The level of statistical significance was set at $p \leqslant 0.05$. Bonferroni correction was applied considering the realization of multiple statistical tests when a statistical significant association was observed in the primary analyses.

3. Results

We recruited 69 FM patients and 61 controls (all female). Age was similar between groups (44.5 ± 6.4 years in FM and 44.0 ± 6.7 in controls). The clinical data of FM patients are described in Table 1. The FM patient had in general long disease duration, almost half were using antidepressant drugs (in doses for treatment of depression) and the overall impact of FM on quality of life, measured by FIQ, was high (70.2 ± 17.8). Most FM patients had at least some level of depression (82.5% by BDI and 87.0% by HDRS), and a considerable percentage had moderate or severe/extreme depression (46.3% by BDI and 58% by HDRS). The women in the control group were not using analgesic or

Table 1
Clinical and therapeutic characteristics of fibromyalgia group.

	Fibromyalgia group (n = 69)
Age (years), mean ± SD	44.5 ± 6.4
Duration of FM (years), mean ± SD	8.5 ± 6.5
FIQ, mean ± SD	70.2 ± 17.8
Regular physical activity	19 (27.5%)
Anti-inflammatory drugs use	8 (11.6%)
Antidepressive use	31 (44.9%)
Level of depression (n, %)	
BDI	
Normal	12 (17.4%)
Mild mood disturbance	14 (20.3%)
Borderline clinical depression	11 (15.9%)
Moderate depression	18 (26.0%)
Severe depression	10 (14.5%)
Extreme depression	4 (5.8%)
HDRS	
Normal	9 (13.0%)
Mild depression	20 (29.0%)
Moderate depression	16 (23.2%)
Severe depression	12 (17.4%)
Very severe depression	12 (17.4%)

SD: standard deviation; FIQ: Fibromyalgia Impact Questionnaire; BDI: Beck Depression Inventory; HDRS: Hamilton Depression Rating Scale.