Association of Markers of Oxidative Stress, Medication Therapy and Life Habits in Fibromyalgia

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Abstract

Objective: To evaluate the association among genetic polymorphisms of glutathione S-transferase, oxidative stress, lifestyle habits and medication therapy in patients with fibromyalgia (FM).

Patients and methods: A total of 187 women (22-84 years) were studied: 81 patients with fibromyalgia (G1) and 106 individuals without the disease (G2). They had their peripheral blood sample submitted for analysis of GSTM1 and GSTT1 polymorphisms, serum levels of thiobarbituric acid reactive substances (TBARS) and trolox equivalent antioxidant capacity (TEAC). All subjects were asked to complete a questionnaire for data registry.

Results: M1/T1 prevalent genotype in G1 (50.6%) and G2 (45.3%; \( P = 0.564 \)). Serum TBARS values were similar between the groups (G1 = 218.0 nmol/L; G2 = 233.44 nmol/L, \( P = 0.9939 \)), whereas TEAC showed reduced values in G1 (2.1 nmol/L) compared with G2 (2.31 nmol/L, \( P = 0.0005 \)). There was a positive correlation between TBARS and TEAC only among patients (\( r = 0.49; P = 0.0019 \)). High levels of TEAC stood out among controls, regardless of smoking or drinking habits, compared with G1 (\( P < 0.0001 \) for both). There was no association between medication and levels of TBARS and TEAC among the analysed patients (\( P > 0.05 \)). However, TEAC, when analysed separately, was lower among patients (\( P = 0.0005 \)).

Conclusions: There was no association of medication and the GSTM1 and GSTT1 polymorphisms with FM. However, reduced TEAC levels in patients reflect the inflammatory effect of the disease, with possible implications in the pathophysiology of FM.

Keywords

Fibromyalgia, Oxidative stress, Reactive oxygen species (ROS), Medication therapy

Introduction

Fibromyalgia (FM) is a syndrome characterized by chronic diffuse musculoskeletal pain and other symptoms that have a negative impact on the quality of life of patients [1,2]. FM predominantly affects women, accounting for approximately 85% of cases [3].

Recently, oxidative stress has been implicated as a relevant event in the pathogenesis of FM [4,5]. Stress is associated with overload of reactive oxygen species (ROS), which damage biomolecules and cellular components [5,6], they trigger the oxidation process [7].

In this context, increased ROS levels have been observed in blood mononuclear cells in patients with FM, followed by reduction by the action of antioxidants, which reflects in changes in markers of oxidative stress, including thiobarbituric acid reactive substances (TBARS) and the Trolox equivalent antioxidant capacity (TEAC), whose level in plasma, serum or cell lysate have been widely used in the assessment of lipid peroxidation [8]. This mechanism leads to oxidative destruction of polyunsaturated fatty acids, which are constituents of cellular membranes [8]. Additionally, glutathione S-transferases, represented by genetic polymorphisms (GSTM1 and GSTT1), act in the detoxification of numerous products of oxidative reactions that damage nucleic acids, lipids and proteins [8]. Glutathione enzyme becomes inactive towards nullity of these genes and with increased exposure of their carriers to oxidative stress [9].

However, there are still controversies over the relationship of FM symptoms with oxidative stress and lipid peroxidation, confirmed by some authors [4,8,10], and denied by others [1]. Thus, genetic factors and their interaction with environmental factors should be identified [2]. This study aimed to evaluate the association of genetic...
polymorphisms GSTM1 and GSTT1, serum levels of TBARS and TEAC, lifestyle habits and medication therapy in patients with FM.

Patients and Methods

Patients

A total of 187 mixed-race women were studied, divided into two groups: G1- 81 women aged 22-72 years (47.0 ± 25.0 years) with FM diagnosis according to ACR [11]; G2 - 106 women aged 22-84 years (53.0 ± 31.0 years) without FM, chronic and systemic diseases under medication treatment. G1 were examined and selected at the Outpatient Pain Clinic and the G2 at the Blood Centre, both in the Hospital de Base University Medical Centre (HB) at the Medical School of São José do Rio Preto (FAMERP). All men and those women under psychiatric medication treatment (antidepressants, benzodiazepines and others) and with chronic disease were excluded. All participants were informed of the study and signed an Informed Consent Document.

Methods

All subjects completed a questionnaire about clinical data and lifestyle habits (smoking and alcohol consumption).

Peripheral blood samples were collected from all subjects for analysis of genetic polymorphisms (GSTM1 and GSTT1). Genomic DNA was extracted from whole blood (5 mL), collected with EDTA using the salting out method [12]. Amplification of polymorphic fragment was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Each reaction included 0.5 µL of each deoxynucleotide (0.8 mM); 2.5 µL of 10 X PCR buffer; 2.5 µL of 10% dimethyl sulfoxide; 2.5 µL of each primer (2.5pM); 0.2 µL of Taq polymerase (5U/µL); 11 µL of Milli Q water; 2 µL of dilution of genomic DNA (0.2 µg). The primers used were: GSTM1 - P1: 5’ AAC TCC CTA AAA GCT AAA C 3’; P2: 5’ GTT GGG CTC CAA TAT ACG GTG G 3’; GSTT1 - P1: 5’ TCG TCT ACT CCT GCT CAC ATC ATC C 3’; P2: 5’ TCA CCG CCG ATC ATG GCC AGC A 3’; T1 alleles (M1/_, M1/T1, T1/_ and _/_). The correlation between the levels of TBARS and TEAC was calculated using the Sperman’s correlation coefficient. Level of significance was set at P < 0.05. Stats Direct, MiniTab14 and GraphPad3 programs were used.

Biochemical analyses of TBARS and TEAC were performed in 40 subjects of G1 and 54 subjects of G2 at random. The TBARS assay of malondialdehyde (MDA) with thiobarbituric acid (TBA), the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), was performed according to Khon and Liversedge [14] and modified by Percário [15]. The post-PCR product was subjected to electrophoresis on 1.5% agarose gel under a constant current of 150 volts for 45 minutes, separating fragments of 423 base pairs (bp) (GSTM1) and 310 bp (GSTT1) using the salting out method [12]. Amplification of polymorphic fragment was obtained at 94°C for 4 minutes, followed by 39 cycles at 94°C for 2 minutes, 59°C for 1 minute and extension at 72°C for 1 minute and a final cycle at 72°C for 10 minutes [13].

Table 1: Distribution of Glutathione S-transferase M1 and T1 polymorphisms (GSTT1 and GSTM1) in patients with fibromyalgia (G1) and controls (G2).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>G1 (N = 81)</th>
<th>G2 (N = 106)</th>
<th>P Value</th>
<th>OR</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>_/ _</td>
<td>23 (28.3%)</td>
<td>39 (36.8%)</td>
<td>0.2928</td>
<td>0.6813</td>
<td>0.3650-1.271</td>
</tr>
<tr>
<td>M1/ _</td>
<td>10 (12.3%)</td>
<td>10 (9.4%)</td>
<td>0.6894</td>
<td>1.352</td>
<td>0.5341-3.423</td>
</tr>
<tr>
<td>M1/T1</td>
<td>41 (50.6%)</td>
<td>48 (45.3%)</td>
<td>0.5646</td>
<td>1.239</td>
<td>0.6936-2.212</td>
</tr>
<tr>
<td>_/ T1</td>
<td>7 (8.6%)</td>
<td>9 (8.4%)</td>
<td>0.9707</td>
<td>1.020</td>
<td>0.3628-2.865</td>
</tr>
</tbody>
</table>

Table 3 shows the analysis of serum with similar TBARS values between G1 (median = 218.0 nmol/L) and G2 (233.44 nmol/L, P = 0.9939), TEAC levels were lower among G1 (median = 2.1 nmol/L), compared with G2 (median = 2.3 nmol/L, P = 0.0005). Figure 2 shows correlation values between TEAC and TBARS, being positive among G1 (r = 0.49; P = 0.0019) and negative among G2 (r = -0.26; P = 0.05).

Figure 1: Photo of 1.5% agarose gel with standard M/T genotypes (Column 1), M/M (Column 2), 0/0 (Column 3) and T/T (Column 4 to 6). A sample of 100-1000pb (M) molecular weight marker was applied in the last column (ladder); bp = base pairs.
medication (median = 251.4 nmol/L), compared with group under two or more medications (183.2 nmol/L), no significant difference (P = 0.345) could be observed.

The analysis of biochemical profile and lifestyle habits (smoking and alcohol consumption) showed similar TBARS values between both groups (P > 0.05), as well as significantly increased levels of TEAC among non-smoking G2 (2.3 nmol/L) and non-drinkers G2 (2.3 nmol/L), compared with G1 (2.0 nmol/L and 2.1 nmol/L; respectively, P < 0.0001 for both; Figure 3).

### Discussion

In this study, GSTs polymorphisms (M1 and T1) involved in biotransformation and elimination of xenobiotics and protection of cells against oxidative stress [20] showed similar distribution between G1 and G2. It is noteworthy that the null genotype, related to higher exposure to oxidative stress due to homozygous deletion of GSTT1 and GSTM1, showed low frequency in both groups, as observed in studies about cancer in Brazilian populations [21]. The same results were found in studies about multiple sclerosis [22] and endometriosis [23,24] with other populations.

Admittedly, oxidative stress depends on the balance between the production of free radicals and the action of antioxidants [25,26]. In this sense, the antioxidant capacity was analysed by means of serum levels of TEAC, which were reduced in G1 compared with G2. This may be a reflection of the FM physiopathological process itself, which produces increased oxidative stress, thereby reducing the

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>G1</th>
<th>G2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1/GSTT1</td>
<td>(N = 9)</td>
<td>(N = 48)</td>
<td>(N = 24)</td>
</tr>
<tr>
<td><strong>T1/—</strong></td>
<td>2</td>
<td>22.2</td>
<td>16</td>
</tr>
<tr>
<td><strong>M1/—</strong></td>
<td>2</td>
<td>22.2</td>
<td>3</td>
</tr>
<tr>
<td><strong>M1/T1</strong></td>
<td>5</td>
<td>55.5</td>
<td>24</td>
</tr>
<tr>
<td><strong>—/—</strong></td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

GSTM1= Glutathione S-transferase M1, GSTT1= Glutathione S-transferase T1, _= nullity, P = Level of significance < 0.05.

<table>
<thead>
<tr>
<th>Serum Levels</th>
<th>G1 (N = 52)</th>
<th>G2 (N = 55)</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/L) Mean</td>
<td>300.66</td>
<td>280.95</td>
<td>0.7123</td>
</tr>
<tr>
<td>± SD</td>
<td>320.21</td>
<td>227.39</td>
<td>0.7123</td>
</tr>
<tr>
<td>TEAC (nmol/L) Mean</td>
<td>1.93</td>
<td>2.31</td>
<td>0.0005</td>
</tr>
<tr>
<td>± SD</td>
<td>0.71</td>
<td>0.21</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

N= Number of subjects, SD = Standard Deviation, P = Level of significance < 0.05.

![Figure 2](image.png)

**Figure 2:** Correlation coefficient between serum thiobarbituric acid reactive substances (TBARS) and trolox equivalent antioxidant capacity (TEAC) in patients with fibromyalgia (G1) and individuals without the disease (G2).
levels of TEAC and TBARS, as observed in the positive correlation between TBARS and TEAC among G1. In contrast, in a similar study of osteoarthritis (another rheumatologic condition associated with inflammatory process), increased levels of TBARS and reduced TEAC [27] were observed. Despite conflicting results, reduced oxidative capacity, whether absolute (genetically determined) or relative (secondary to increased free radical production due to the inflammatory process) is likely to participate in FM pathophysiology [4,26,27].

Medication therapy for pain in FM, although lacking systematised clinical management protocols, includes the use of several classes of medications, namely anti-inflammatory, analgesics and muscle relaxants [28]. These drugs relieve pain and often improve the functional capacity of the patient. The possible antioxidant role of these medications or an association between them, however, is disputable [24-27]. This study has not shown statistically significant association between medications used and serum levels of TBARS and TEAC, possibly due to the small sample size. However, studies have observed the influence of analgesics on levels of TBARS [25]. Furthermore, this study also found that lifestyle habits, such as smoking and alcohol consumption, were similar among G1 and G2, in agreement with current literature data [29]. However, higher levels of TEAC in non-smokers and non-drinkers among G2 suggest greater protection against oxidative stress in these individuals.

In conclusion, GSTM1 and GSTT1 polymorphisms were not associated with FM patients in this study. There was no association between medications and genetic variants or TBARS and TEAC levels among the analysed patients. However, lower levels of TEAC in non-smokers and non-drinkers among G2 suggest greater protection against oxidative stress in these individuals.

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Ethical Statement

This study was approved by the Research Ethics Committee of the Medical School of São José do Rio Preto /SP (CAEE: 14187413.2.0000.5415).

References