Evaluation of vitamin D status in a population with Fibromyalgia

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Summary

RESUMO

Introdução: A fibromialgia, uma doença crónica caracterizada por dor músculo-esquelética generalizada, está entre as condições associadas à deficiência de vitamina D, apesar de não ter sido encontrado ainda um mecanismo biológico claro para esta associação. A evidência disponível na literatura é controversa, existindo estudos que referem uma maior prevalência de deficiência de vitamina D em doentes com fibromialgia e outros que revelam não haver diferenças.

Objectivos: O objectivo do nosso estudo consistiu na avaliação dos níveis de 25-hidroxivitamina D em doentes com fibromialgia e em controlos não afectados. Avaliámos ainda a relação entre os níveis de vitamina D, o limiar e intensidade da dor e a severidade da doença.

Métodos: No nosso estudo transversal, avaliámos os níveis sanguíneos de 25-hidroxivitamina D em 22 doentes com o diagnóstico de fibromialgia e nas suas 22 irmãs sem a doença. A avaliação laboratorial foi feita no mesmo dia para o doente e para o controlo emparelhado. A recolha dos dados decorreu durante o período de dois meses no Outono/Inverno. Todos os doentes e controlos foram sujeitos ao exame físico (incluindo a avaliação do limiar doloroso) e preencheram a versão portuguesa do Fibromyalgia Impact Questionnaire.

Resultados: Os níveis médios de 25-hidroxivitamina D no soro foram de 18,53±6,47ng/ml no grupo de doentes com fibromialgia e 15,61±5,05ng/ml no grupo de controlo. Não foram encontradas diferenças entre os grupos no que respeita aos níveis médios de vitamina D nem à classificação em deficiência de vitamina D (<20ng/ml), insuficiência (21-29ng/ml) e suficiência (>30ng/ml). Nos doentes com fibromialgia foi encontrada uma associação negativa entre os níveis de vitamina D e a pontuação no Fibromyalgia Impact Questionnaire (r=-0,554; p=0,009), bem como entre estes e a intensidade da dor medida pela Escala Visual Analógica (r=-0,447; p=0,042). Não foi encontrada relação entre os níveis de vitamina D e o limiar doloroso medido pelo dolorímetro. 95,2% da população estudada tinha níveis deficientes/ insuficientes de vitamina D.

Conclusões: A deficiência de vitamina D não foi encontrada mais frequentemente nos pacientes com fibromialgia do que nos controlos sem a doença. Nos doentes com fibromialgia foi encontrada uma associação negativa entre os níveis de vitamina D, a severidade da doença e a intensidade da dor medida pela Escala Visual Analógica. Não foi encontrada relação entre os níveis de vitamina D e o limiar doloroso medido através de dolorímetro. O nosso estudo mostrou uma alta prevalência de deficiência/insuficiência de vitamina D na nossa população.

Palavras-chave: fibromialgia, vitamina D, limiar doloroso

ABSTRACT

Background: Fibromyalgia, a condition characterized by chronic widespread musculoskeletal pain, is among the chronic pain conditions that have been associated with vitamin D deficiency, though no clear biological mechanism has been yet found. The results of studies are controversial, existing studies reporting a higher prevalence of vitamin D deficiency in patients with fibromyalgia, with others reporting no differences.

Objective: The aim of our study was evaluate serum 25-hydroxyvitamin D levels in patients with fibromyalgia and unaffected controls. We also aimed to evaluate the relation between 25-hydroxyvitamin D levels, pain threshold, pain intensity and the severity of the disease.

Methods: In our transversal study we measured serum 25-hydroxyvitamin D in 22 patients with a diagnosis of fibromyalgia and 22 matched-controls (unaffected sisters). The blood test was performed in the same day for patient and matched-control. All the data was collected within 2 months in Autumn/Winter. All patients and controls underwent physical examination (including evaluation of pressure-induced pain threshold) and answered Fibromyalgia Impact Questionnaire.

Results: We found a mean serum 25-hydroxyvitamin D level of 18,53±6,47ng/ml in the fibromyalgia group and 15,61±5,05ng/ml in the control group. No statistically significant differences were found between groups regarding mean serum vitamin D levels or the

classification in vitamin D deficiency (<20ng/ml), insufficiency (21-29ng/ml) and sufficiency (>30ng/ml). In the fibromyalgia group, we found a negative association between vitamin D level and Fibromyalgia Impact Questionnaire score (r=-0,554; p=0,009), as well as with pain intensity measured by Visual Analog Scale (r=-0,447; p=0,042). No relation was found between vitamin D levels and pressure-induced pain threshold. 95,2% of our population were vitamin D deficient/insufficient.

Conclusions: Vitamin D deficiency was not more prevalent in fibromyalgia patients than in the control group without the disease. We found a negative association between low vitamin D levels, the severity of the disease (as assessed by FIQ) and pain intensity measured by Visual Analog Scale, but no relation was found with pressure-induced pain threshold. Our study showed a high prevalence of vitamin D deficiency/insufficiency in our population.

Keywords: fibromyalgia, vitamin D, pain threshold

Introduction

Fibromyalgia (FM) is a common condition characterized by chronic widespread pain for more than three months and by the presence of tenderness on pressure in at least 11 out of 18 defined points, according to the 1990 American College of Rheumatology criteria. These symptoms are frequently associated with a variety of other symptoms including morning stiffness, sleep disorders, fatigue, anxiety and depression, with negative impact in quality of life[1].

The prevalence of FM in general population was estimated at between 0.5% and 5%, disproportionably affecting women[2]. Despite the high prevalence of FM (approximately 3,6% of the population in Portugal – 1.8% in men and 5.2% in women)[3], treatment remains complex.

Chronic pain is among the many conditions that have recently been associated with vitamin D deficiency[4] and a number of studies have suggested a link between low levels of vitamin D and higher incidence of chronic pain[5-15], but only one study reported this association in patients with FM[16]. Some reports suggest that vitamin D supplementation may have an analgesic effect[17-19]. There are no studies about the relation of vitamin D with pressure-induced pain threshold. Altogether, the available evidence does not allow the conclusion that vitamin D is relevant in chronic pain, with studies reporting a negative association[20-28]. All the authors agree that we need better evidence.

The pathophysiology of FM remains unclear but several mechanisms may be involved, such as abnormalities in Central Nervous System (CNS) sensory processing (central sensitization, dysregulation of descending inhibitory pathways and peripheral sensitization),

mood and peripheral tissue abnormalities, such as ragged red fibers, inflammatory infiltrates, prolonged muscle tension and ischemia, with changes in muscle pH[29-31].

It has been suggested that abnormalities in muscle tissue may induce an increased responsiveness of neurons innervating these tissues. This would contribute to an increased tonic nociceptive input in FM, resulting in central sensitization through mechanisms related with the production of nitric oxide (NO) and consequent hyperexcitability of Pain Transmission Neurons (PTNs). Glial cells activated by painful stimuli also play a role in signaling modulation, enhancing the excitability of PTNs through the release of NO and Radical Oxygen Species (ROS)[29, 32-35].

Vitamin D, in addition to its well known functions in calcium-phosphate metabolism, exerts a variety of biological actions that can be related with the origin of pain in FM, namely through influences upon muscle function and pain perception.

Through Vitamin D Receptors (VDR) present in skeletal muscle tissue, this vitamin influences de novo protein synthesis involved in muscle cell contractility, proliferation, and differentiation[36-37]. In fact, several observational studies and randomized controlled trials suggest a positive association between vitamin D levels and muscle strength and function[38-42].

VDR also have been identified in neurons and glial cells. Vitamin D was found to be neuroprotective against damage induced by ROS, through the inhibition of the inducible NO synthase[43]. It was suggested that vitamin D may influence pain perception by promoting the descending CNS pathways that inhibit musculoskeletal pain and downregulating the transcription of calcitonin gene, which has direct analgesic effect especially in bones[43-44].

Vitamin D has been related with “winter depression” or seasonal affective disorder (SAD) and low levels of vitamin D were positively associated with depression and anxiety in FM patients. FM patients have the tendency to remain indoors and to be less physically active, thus contributing to less sun exposure and lower levels of vitamin D[43, 45-47].

Osteomalacia, the tip of the iceberg of vitamin D deficiency, is a differential diagnosis of fibromyalgia.

The main aim of our study is to verify the prevalence of vitamin D deficiency among FM patients, in comparison with their unaffected sisters. Another goal of our study is to study the relationship between vitamin D status, pressure-induced pain threshold and pain intensity. We also want to ascertain if there is any linkage between severity of FM (measured by Fibromyalgia Impact Questionnaire) and the corresponding vitamin D serum concentrations.

Materials and methods

Study population

In order to address the hypothesis described above we decided to study female patients with fibromyalgia paired with an unaffected sister.

Participants were drawn from a list of 712 patients with an established diagnosis of FM from a single site (all diagnosed and followed by Prof. J.A.P. da Silva). The following screening criteria were used for selection: Female gender, age between 18 and 55 years of age, absence of any other chronic pain condition, residence within a radius of 100 Km from the study centre. Selected patients were contacted by phone and asked to participate if: 1. They had at least one unaffected sister also willing to participate, 2. The mother of both was the same person, still alive and capable of participating and providing reliable information, and 3. All the family members were willing to travel to the research site and participate in the study, which involved signing an informed consent, responding to questionnaires, providing a blood sample and undergoing physical examination. Participants were reimbursed for transportation costs but no other fees were offered. A total of 22 patients and 22 controls were included in the study.

The study was approved by Ethical Committee of the Faculty of Medicine of the Universidade de Coimbra before the onset of the study.

All research proceedings were performed in the morning. After receiving an explanation of the study procedures and having an opportunity to present any questions and discuss all issues, participants signed an informed consent form. This was followed by a fasting blood

sample collection. Breakfast was offered to participants before the other procedures were started.

Interview and Fibromyalgia Impact Questionnaire

This study involved a 9 investigators team, working together in the data collection.

All the patients and controls were subjected to a demographic questionnaire, which included age, country of birth, marital status, years of education, disease duration, medication and co morbidities. All the controls were asked about widespread pain longer than 3 months, and if the answer was positive, tender points were counted.

All the patients and controls completed the Portuguese version of Fibromyalgia Impact Questionnaire (FIQ-P) as a part of the interview. FIQ is a well-validated tool for the evaluation of status, progress and outcome in FM[48]. It has been designed to measure the components of health status that are believed to be most affected by FM. The FIQ is composed of 10 questions. The first question contains 11 items related to the ability to perform large muscle tasks - each question is rated on a 4 point Likert type scale. Items 2 and 3 ask the patient to mark the number of days they felt well and the number of days they were unable to work (including housework) because of fibromyalgia symptoms. Items 4 through 10 are horizontal linear scales marked in 10 increments on which the patient rates work difficulty, pain (VAS), fatigue, morning tiredness, stiffness, anxiety and depression. FIQ is scored in such a way that a higher score indicates a greater impact of the syndrome on the person. Each of the 10 items has a maximum possible score of 10, thus the maximum possible score is 100[48]. The average fibromyalgia patient scores about 50; severely afflicted patients are usually 70 plus[49].

Physical Examination

All the patients and controls had their height, weight and body mass index (BMI) measured, as well as pressure-induced pain threshold at five different locations: a) right and left tibial midpoint, b) sternal manubrium, and c) right and left second finger nail bed. Pain threshold was measured with a pressure algometer (dolorimeter) twice in each site and the medium value was calculated. Pressure was applied at the referred sites until the patient verbally expresses pain and the result was expressed in kg, until a maximum of 10kg.

Nine different researchers saw the subjects and performed the physical examination.

Serum Vitamin D measurement and interpretation

All the venous blood samples, from all the patients and controls, were taken between November 2010 and January 2011, which corresponds to the autumn/winter season in Coimbra (40º15'N), and the matched-control blood sample was collected in the same day as the patients', thus controlling for seasonal variations. Serum was extracted by centrifugation at 5000 rpm for 10 minutes and stored frozen at -20ºC until analysis, which was performed less than a month after sample collection. Serum 25-hydroxyvitamin D (25(OH)D) was measured by a chemiluminescence immunoassay - DiaSorin LIAISON® - in the Hormonology Laboratory of Hospitais da Universidade de Coimbra. Serum 25(OH)D levels are expressed in ng per milliliter.

Although there is no consensus on optimal 25(OH)D levels, we defined vitamin D deficiency as a 25(OH)D level of less than 20 ng per milliliter (50nmol/L), insufficiency as a level between 21 and 29 ng per milliliter (52-72nmol/L), and a level higher than 30 ng per

milliliter (75nmol/L) was considered to indicate vitamin D sufficiency, as in agreement with most experts[4, 50-51].

Statistical analysis

All the data was stored using Microsoft® Office Excel® 2007 and analyzed by SPSS 18.0.

For continuous variables, the descriptive statistics included mean, median, range, minimum and maximum, standard deviation and variance. For nominal variables, descriptive statistics included frequency and percent.

We tested the normality of the distribution using Kolmogorov-Smirnov test in order to apply the more adequate statistical test. Homogeneity of variances was considered using Levene's test.

Student's t-test was used in continuous variables which satisfied the required normality assumptions and Mann-Whitney test in continuous variables which didn't satisfy the normality assumptions. In order to verify the association between variables, we used bivariate correlation of Pearson or Spearman (depending on the existence of normality of the variables). To verify possible factor interferences in the variables relation, we used general linear model (GLM) ANCOVA.

The minimum significance level to reject null hypothesis, in all used statistic tests, was set to 0,05.

Results

Socio-demographic characteristics

We had 44 participants in our study (22 patients with fibromyalgia paired with their unaffected sisters); one pair was excluded from the analysis because both patient and sister had fibromyalgia criteria, thus not filling the inclusion criteria. Socio-demographic characteristics are summarized in table 1.

			FM Group	**Control Group**	**p**
Sex	**Female**	Count (%)	**21 (100,0%)**	**21 (100,0%)**	
Age (years)		Mean±SD	**40,95±10,40**	**40,14±10,32**	0,801
Birth Country	**Portugal** **Brazil**	Count (%)	**21 (100,0%)** **0 (0,0%)**	**20 (95,2%)** **1 (4,8%)**	0,311
Years of Education		Mean±SD	**12,62±4,18**	**12,05±5,36**	0,702
Number of Children		Mean±SD	**1,24±0,94**	**1,19±1,03**	0,877
Marital Status	**Single** **Married** **Divorced**	Count (%)	**4 (19,0%)** **17 (81,0%)** **0 (0,0%)**	**7 (33,3%)** **12 (57,1%)** **2 (9,5%)**	0,159
Body Mass Index (kg/m^2)		Mean±SD	**25,55±2,99**	**26,26±4,89**	0,574
	Normal **Pre-Obese** **Obese Class I** **Obese Class II**	Count (%)	**10 (47,6%)** **10 (47,6%)** **1 (4,8%)** **0 (0,0%)**	**9 (42,9%)** **8 (38,1%)** **2 (9,5%)** **2 (9,5%)**	0,456

Table 1 – Socio-demographic characteristics

All the 42 participants in our study were female, and the mean age was 40,95±10,40 years old for the FM group and 40,14±10,32 for the control group. No statistically significant differences were found between groups regarding socio-demographic characteristics. None of the participants were under vitamin D supplementation.

Pain threshold

As expected, we verified that pressure-induced pain threshold was lower in the FM patients in all the tested locations, as shown in Table 2.

	Tibia midpoint		**Sternal manubrium**		**Nail bed**		**Mean Pain Threshold**	
	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
FM group	3,01±1,60	0,002	1,91±1,45	0,004	3,13±1,76	0,005	2,68±1,45	0,001
Control group	4,68±1,66		3,16±1,20		4,90±1,89		4,24±1,32	

Table 2 – Comparison of Pain Threshold between the FM Group and the Control Group. Pain Threshold was lower for the FM group in all the tested locations.

FIQ score

FIQ score was higher in FM patients than in control group. The mean±SD FIQ score was 49,61±13,23 in FM group against 18,56±14,75 in the control group. These differences are statistically significant, verified with Mann-Whitney U test (U=32,000; p=0,000). Figure 1 shows the mean FIQ scores in each group.

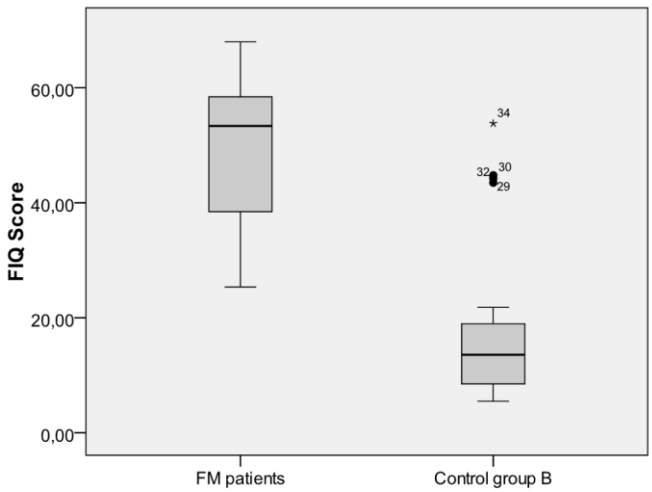

Figure 1 – FIQ Score in FM and Control group.

Vitamin D Status

In the general population, the mean serum 25(OH)D was 17,08±5,92ng/ml, ranging from 5,40 to 32,20ng/ml. We verified that 76,2% (n=32) had vitamin D deficiency, 19,0% (n=8) had vitamin D insufficiency and only 4,8% (n=2) had vitamin D sufficiency.

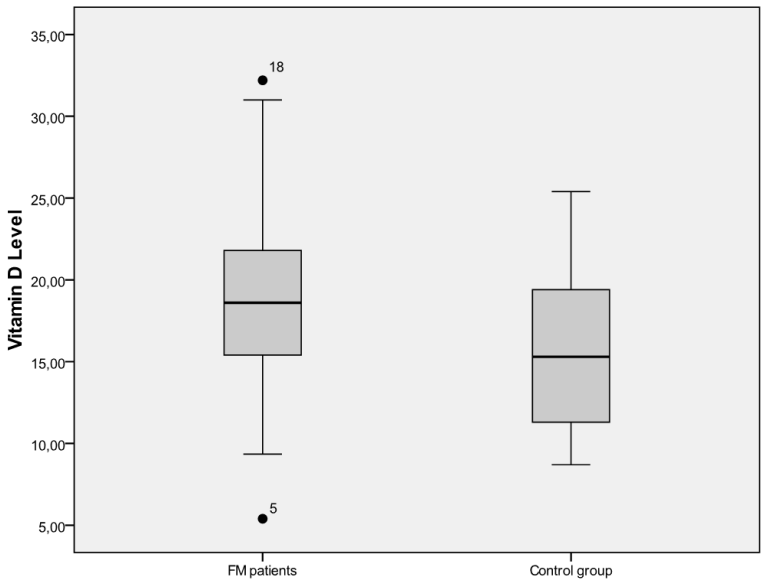

Figure 2 – Mean serum 25(OH)D levels in FM Group and Control Group: 18,53ng/ml and 15,61ng/ml, respectively

Figure 2 shows the mean serum 25OHD levels in the FM group and in the control group. Mean serum 25(OH)D was 18,53±6,47ng/ml (5,40-32,20) in the FM group and 15,61±5,05ng/ml (8,70-25,40) in the control group. Although mean 25(OH)D levels were higher in the FM group, the difference is not statistically significant (t=1,628; p=0,111). We found that there were no statistically significant differences between the paired means of the FM patient and her sister, but there was no correlation regarding vitamin D levels between the FM patient and the matched-control (r=0,061; p=0,794) (*Appendix 4*)

	Vitamin D Deficiency	Vitamin D Insufficiency	Vitamin D Sufficiency
FM patients	15 (71,4%)	4 (19.0%)	2 (9.5%)
Control group	17 (81,0%)	4 (19.0%)	0 (0%)
Total	32 (76,2%)	8 (19.0%)	2 (4,8%)

Table 3 – Classification regarding vitamin D status in the general population, FM Group and Control Group

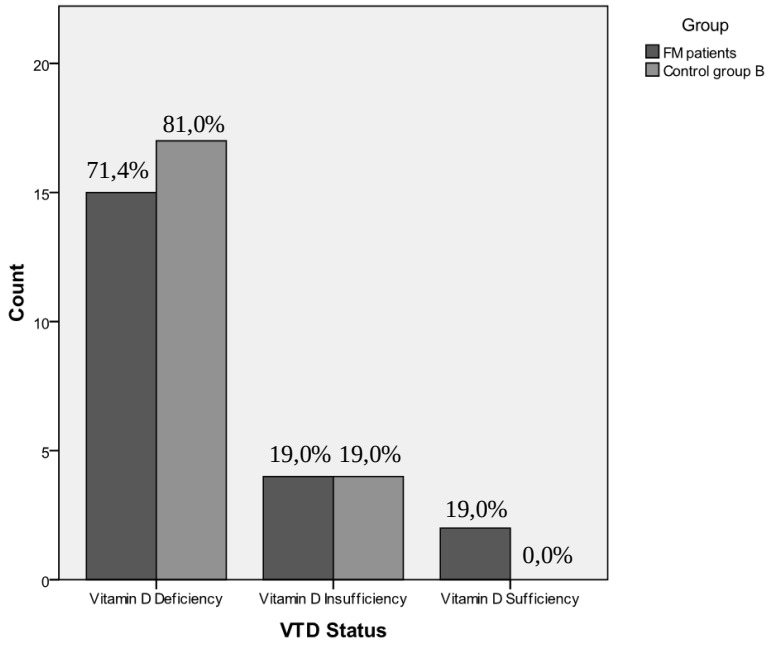

Figure 3 - Graphic showing the distribution of Vitamin D Deficiency, Insufficiency and Sufficiency in Control Group and FM Group

Table 3 and figure 3 show the classification in vitamin D deficiency, insufficiency or sufficiency. There were no statistically significant differences between the groups regarding this classification (χ^2=2,125; p=0,346).

Vitamin D and Pain Threshold

Pain Threshold	Serum 25(OH)D	
	r / rho	**p**
Tibial midpoint	-0,022 (r)	0,890
Sternal manubrium	-0,082 (r)	0,605
Second finger nail bed	-0,049 (rho)	0,757
Mean 3 sites	-0,058 (r)	0,715

Table 4 – Pearson/Spearman correlation between Pain Threshold and serum 25(OH)D levels

Using Pearson correlation (r) and Spearman's rank correlation coefficient (rho) we found that there is not significant association between serum 25(OH)D and mean pain threshold ($r<0,30$; $p>0,05$). The General Linear Model (ANCOVA) showed that the variable "group" interacted with the results in pain threshold ($p\leq0,05$; Power >0,500), while the variable "age" only interacted with pain threshold in sternal manubrium ($p>0,05$ but Power>0,500).

	FM Group		**Control Group**	
	r/rho	p	r/rho	p
Tibial Midpoint	**0,261**	0,253	**-0,076**	0,742
Sternal Manubrium	**0,079**	0,735	**-0,047**	0,840
Second finger nail bed	**0,243**	0,288	**-0,224**	0,330
Mean Pain Threshold	**0,244**	0,286	**-0,153**	0,509

Table 5 – Correlation between pain threshold and 25(OH)D levels in FM Group and Control Group

We verified that there is no significant correlation between serum 25(OH)D and pressure-induced pain, neither in the FM group nor in the Control Group. Although the differences are not statistically significant, it is remarkable that in the FM group we obtained a positive correlation while in the control group this correlation is negative.

Pain Intensity (VAS)	Serum 25(OH)D	
	r / rho	**p**
FM Group	-0,447 (r)	0,042
Control Group	-0,321 (rho)	0,156

Table 6 – Correlation between Pain Intensity and 25OHD level

As shown in table 6, we found a statistically significant negative association in the FM group between vitamin D levels and pain intensity measured by Visual Analog Scale (VAS) (r=-0,447; p=0,042), while in the control group this correlation was also negative but not statistically significant (rho=-0,321; p=0,156).

Vitamin D and FIQ score

In our study, no statistically significant correlation was found between 25(OH)D levels and FIQ in our overall population. However, analyzing the groups separately, we found a statistically significant negative association between serum 25(OH)D and FIQ score (r=-0,554; p=0,009), while in the control group this association is negative but not significant (rho=-0,183; p=0,427).

	r / rho	**p**
Vitamin D/ FIQ General Population	- 0,039	0,807
Vitamin D/ FIQ FM Group (r)	-0,554	0,009
Vitamin D/ FIQ Control Group (rho)	-0,183	0,427

Table 7 – Correlation between FIQ Score and 25(OH)D levels

Discussion

Several reports suggested that vitamin D may have a role in chronic widespread pain syndromes including fibromyalgia[7, 10, 13, 15-16, 47], but this is controversial, existing other studies not confirming these findings and others showing no association[21-22, 26, 28]. These contradictory results may be related to several potentially problematic methodological issues that may have affected the results.

Plotnikoff and Quagley found that 93% (n=140) of subjects with chronic musculoskeletal pain had deficient levels of vitamin D (mean 12,08ng/ml), but their study did not have a control group and none of the patients had the diagnosis of fibromyalgia as defined by ACR[13]. If in our study we didn't have a control group, we would have obtained similar results as Plotnikoff and Quagley - a high prevalence of vitamin D deficiency in the FM group - thus strengthening the importance of the existence of a control group.

Chronic pain/FM patients were found to have lower levels of 25(OH)D compared with other rheumatology outpatients. However, the control group was composed by patients with other chronic painful conditions and 95% of the patients in the "osteoporosis-osteopenia group", who functioned as a control group, were receiving vitamin D treatment, thus confounding the results [15]. Al-Allaf *et al* in 2003 reported a significantly greater proportion of FM patients with low 25(OH)D levels in women with FM as compared to age matched controls (45%vs18,9%). However, measurements were taken in March for the patients and in May for controls, despite the known seasonal variability of vitamin D levels. Additionally, their definition of low levels of vitamin D (<8ng/ml)[16] was not in agreement with current concepts. Armstrong *et al* (2007) also reported a high prevalence of vitamin D deficiency and insufficiency (13% and 56% respectively) in 75 patients with the diagnosis of fibromyalgia, but there were no controls in this study[47]. A retrospective study carried out in Mumbai

showed that in 71 patients with low vitamin D levels (<9,9ng/ml), 51% had back pain and 20% had generalized body ache as presenting symptoms. Again, there were no controls[10]. Another study found that 81,7% of patients with chronic low back pain had hypovitaminosis D, compared with 60% of the controls. However, the definition of hypovitaminosis D was too high (<40ng/ml)[12]. A small study in an urban Australian aboriginal population also showed lower levels of 25(OH)D in eight patients with muscle pain (16,35ng/ml) compared with eight patients without (23,3ng/ml).

Our findings do not confirm the association of fibromyalgia with low vitamin D levels. There are no statistically significant differences between the FM group and the control group, and the mean 25(OH)D levels were even numerically higher in the FM group (18,53±6,47ng/ml and 15,61±5,05 respectively; p=0,111). The frequency of vitamin D deficiency/insufficiency was also similar in patients with FM and in healthy controls (χ^2=2,125; p=0,346). We verified that in the FM group, 71,4% (n=15) are vitamin D deficient, 19,0% (n=4) are insufficient and 9,5% (n=2) are sufficient. These results are similar to those verified in the control group: 81,0% (n=17) are vitamin D deficient and 19,0% (n=4) are insufficient, with none having sufficient levels of vitamin D. We employed patients and controls from the same family hoping that this could diminish background differences related to aspects such as education levels, professional activity, food habits, genetic background. This ought to increase our ability to demonstrate actual differences related to FM. In fact, there was no correlation between vitamin D levels in each pair FM patient-unaffected sister suggesting that being part of the same family does not affect vitamin D levels.

Our negative findings are in agreement with the studies presented below.

Block (2004) did not find differences between 69 patients with chronic musculoskeletal pain who fit the ACR criteria for FM and 32 controls (mean serum 25(OH)D 22,6vs24,8ng/ml). However, his control population was not adequate, as it was composed by patients with chronic widespread musculoskeletal pain[26]. Warner and Arnspiger (2008) found no association between low 25(OH)D levels and diffuse musculoskeletal pain in 184 patients, and no improvement of pain was observed after treatment with vitamin D in patients who had low baseline levels. The control group was composed by 104 patients with osteoarthritis[28]. In a study comparing serum 25(OH)D between 68 premenopausal women with FM and 82 age-matched controls, it was demonstrated that a low 25(OH)D level (<20ng/ml) is not more common in women with FM than in controls without the disorder, and the use of different cut off points did not affect the findings. In a logistic regression analysis, the authors failed to show that serum vitamin D had any relationship with the diagnosis of fibromyalgia but they didn't study the relationship with disease severity[22].

In 2010, de Rezende Pena *et al* found no differences in mean serum 25(OH)D between the FM and control group. This study showed a mean 37,51±18,78ng/ml and 38,23±16,18ng/ml in the FM and in the control group, respectively (p=0,78). There were no significant differences between groups regarding the classification in vitamin D deficiency, insufficiency and sufficiency[21]. However, the data collection of the control group occurred predominantly during winter and spring in relation to the patient group (70,62% vs 54%), which may have contributed to lower levels of vitamin D found in the control group, dispelling a possible difference between the groups regarding serum levels of vitamin D. The mean serum 25(OH)D levels in this population were higher than the levels found in our study, probably due to the differences in latitude (26ºS vs 45ºN) and sunlight incidence.

In our research, we have overcome some of the limitations of the studies referred above. We had an adequate control group, composed by matched unaffected sisters (generalized chronic pain was excluded), all patients were white, thus no potential ethnical differences had to be considered, all the measurements were made between November 2010 and January 2011, and patient and matched-control had their serum samples for vitamin D level assessment collected in the same day. Therefore, seasonal variations did not confound our results, contrarily to some studies referred above, and we can conclude that low vitamin D levels are not related with the presence of fibromyalgia.

What about pain and disease impact?

The FIQ score was higher (49,61±13,23 vs 18,56±14,75) and pressure-induced pain threshold was lower (3,01vs4,68 in tibia midpoint; 1,91vs3,16 in sternal manubrium and 3,13vs4,90 in nail bed) in all tested locations in the FM group (table 2), confirming the expected profile for each group and supporting the proper composition of our population.

Using correlation coefficients (Pearson and Spearman), we found no association between levels of vitamin D and the sensitivity to pressure-induced pain in all the tested locations (table 4 and 5), but in the FM group we found a statistically significant negative association between 25(OH)D levels and pain intensity measured by VAS in the FIQ questionnaire (table 6): “In the past week, how bad has your pain been?” . This opens the possibility that vitamin D levels may influence the experience of pain (i.e. suffering) in FM even if it does not change pressure-pain threshold significantly. Besides, the GLM (ANCOVA) showed that the variable “group” (FM/Control) interacted with the results in the correlation between vitamin D levels and pain threshold, suggesting that the influence of Vitamin D upon pain threshold is different in FM and in controls. These results warrant further studies designed to clarify the relation between 25(OH)D and the pain experienced by FM patients.

These findings are not in agreement with the study performed by de Rezende Pena *et al*, who found no association between pain intensity (VAS) and deficient/insufficient 25(OH)D[21].

Straube *et al* (2009), in a review about 22 studies about pain and vitamin D, found no evidence of association between the prevalence of chronic pain and latitude, as well as between chronic pain syndromes and low levels of vitamin D. The authors suggested the need of large, double-blind, randomized control trials. They found that supporting studies are insufficient to support the hypothesis that vitamin D supplementation is a useful treatment for chronic pain[23]. Our results are in agreement with this concept

An interesting finding of our study is a statistically significant negative association between 25(OH)D levels and FIQ score in the FM group (r=-0,554; p=0,009). These results are not in agreement with those verified by Al-Allaf and colleagues, who reported no significant differences in disease or lifestyle parameters (measured by Health Assessment Questionnaire, McAlpine mobility score and FIQ) between FM patients who had high and those who had low serum 25(OH)D levels, although their definition of low 25(OH)D (<8ng/ml) may have interfered with the results[16]. Another study reported no significant correlation between FIQ and vitamin D levels in patients with FM, but their methodology to ascertain this was different from ours. They verified the median FIQ in each vitamin D status class (Deficient, Insufficient, and Sufficient) and they found no differences between classes regarding the median FIQ. However, their classification in vitamin D deficiency (<10ng/ml), insufficiency (10-20ng/ml) and sufficiency (>20ng/ml) are not in agreement with current concepts.

Our results suggest that patients with a higher impact (severity) of FM have lower levels of Vitamin D. This might simply reflect a tendency of the more severely disabled FM patients to

remain indoors thus resulting in lower levels of vitamin D. On the other hand, it is not possible to exclude that the lower levels of vitamin D are causally related to the higher disability. The negative correlation found between 25(OH)D levels and pain intensity measured by VAS in the FM group supports the idea that vitamin D levels may have a role in the severity of FM. This can only be clarified by a prospective randomized trial of vitamin D in FM patients.

One alarming finding of our study was the remarkably high prevalence of vitamin D deficiency/insufficiency in our population. The mean serum level in the general population was 17,08±5,92ng/ml (ranging from 5,40 to 32,20ng/ml). According to the classification accepted by most experts, only 4,8% (n=2) of our population were vitamin D sufficient and we were not expecting such high prevalence of vitamin D deficiency in a population with a mean age of 40,55 years. This finding deserves to be clarified. If Portuguese population has a high level of vitamin D deficiency, we need to pay attention to 25(OH)D serum levels and correct it if necessary (advising for adequate sun exposure and a diet rich in foods naturally rich in vitamin D, such as oily fish), in order to maximize health and reduce the risk of chronic diseases such as hypertension, type-1 diabetes, multiple sclerosis and common cancers[51].

Although there is no absolute consensus on optimal levels of 25(OH)D measured in serum, the definition we used for vitamin D deficiency, insufficiency and sufficiency is in agreement with most experts. Chapuy et al observed that PTH are inversely associated with vitamin D levels until vitamin D reach 30-40ng/ml, at which point PTH levels begin to plateau at their nadir[50]. Based on provocative testing with vitamin D supplementation, it was suggested that vitamin D deficiency should be defined as 25(OH)D < 20 ng/ml. With all this information collectively, most experts consider vitamin D deficiency as a 25(OH)D level <20ng/ml,

vitamin D insufficiency as a level between 21 and 29 ng/ml and the preferred/sufficient vitamin D level as >30ng/ml[4].

The findings of our study made us aware of the possibility that a very high prevalence of vitamin D insufficiency/deficiency is to be found in the Portuguese population with all its health consequences. The data was collected in autumn/winter and Coimbra is located at 45ºN, which may have overestimated our results, and it would be useful to verify if this high prevalence of vitamin D deficiency remains in spring/summer seasons.

The limitations of our study were the difficulty in recruiting FM patients with unaffected sisters with their mother still alive (required in order to get the data needed for all the researchers in this project). The assessment of sun exposure and ingestion of foods rich in vitamin D would have been useful to verify if these are the causes of the high prevalence of vitamin D deficiency and insufficiency. We believe that this lack of data didn't confound our findings in the comparison between groups, because both FM and control group were very similar and homogeneous populations and the data collection occurred in the same day for the patient and the matched control. One possible limitation in the assessment of patients and controls' pain threshold was that nine different researchers performed the physical examination, thus could have been different methods of examination, although we tried to standardize the procedure. Another problem we faced was the hardness in comparing results of studies performed in different areas of the world due to local variations in the prevalence of hypovitaminosis D.

We can conclude that mean levels of 25(OH)D were similar in FM patients compared with an unaffected control group. The prevalence of vitamin D deficiency and insufficiency was also similar in the two groups. No association was found between vitamin D levels and pressure-pain threshold, although we found a negative relation between vitamin D levels and

pain intensity measured by VAS, which deserves further studies. A negative association between FIQ score and vitamin D levels was found. This issue deserves further research in prospective controlled trials.

We found a high prevalence of vitamin D deficiency/insufficiency in the general population (95,2%), warning about a possible poor vitamin D status in the Portuguese population. Although no relationship was found between low levels of vitamin D and fibromyalgia, it is important to evaluate and correct this poor vitamin D status, in the FM patients and in controls.

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References

1. Wolfe, F., et al., *The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee.* Arthritis Rheum, 1990. **33**(2): p. 160-72.
2. White, K.P. and M. Harth, *Classification, epidemiology, and natural history of fibromyalgia.* Curr Pain Headache Rep, 2001. **5**(4): p. 320-9.
3. Branco, J.C., et al., *Prevalence of fibromyalgia: a survey in five European countries.* Semin Arthritis Rheum, 2010. **39**(6): p. 448-53.
4. Holick, M.F., *Vitamin D deficiency.* N Engl J Med, 2007. **357**(3): p. 266-81.
5. Atherton, K., et al., *Vitamin D and chronic widespread pain in a white middle-aged British population: evidence from a cross-sectional population survey.* Ann Rheum Dis, 2009. **68**(6): p. 817-22.
6. Badsha, H., M. Daher, and K. Ooi Kong, *Myalgias or non-specific muscle pain in Arab or Indo-Pakistani patients may indicate vitamin D deficiency.* Clin Rheumatol, 2009. **28**(8): p. 971-3.
7. Benson, J., et al., *Muscle pain as an indicator of vitamin D deficiency in an urban Australian Aboriginal population.* Med J Aust, 2006. **185**(2): p. 76-7.
8. Erkal, M.Z., et al., *High prevalence of vitamin D deficiency, secondary hyperparathyroidism and generalized bone pain in Turkish immigrants in Germany: identification of risk factors.* Osteoporos Int, 2006. **17**(8): p. 1133-40.
9. Gloth, F.M., 3rd, et al., *Can vitamin D deficiency produce an unusual pain syndrome?* Arch Intern Med, 1991. **151**(8): p. 1662-4.
10. Kanekar, A., M. Sharma, and V.R. Joshi, *Vitamin d deficiency-a clinical spectrum: is there a symptomatic nonosteomalacic state?* Int J Endocrinol, 2010. **2010**: p. 521457.

11. Knutsen, K.V., et al., *Vitamin D status in patients with musculoskeletal pain, fatigue and headache: a cross-sectional descriptive study in a multi-ethnic general practice in Norway.* Scand J Prim Health Care, 2010. **28**(3): p. 166-71.

12. Lotfi, A., et al., *Hypovitaminosis D in female patients with chronic low back pain.* Clin Rheumatol, 2007. **26**(11): p. 1895-901.

13. Plotnikoff, G.A. and J.M. Quigley, *Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain.* Mayo Clin Proc, 2003. **78**(12): p. 1463-70.

14. Turner, M.K., et al., *Prevalence and clinical correlates of vitamin D inadequacy among patients with chronic pain.* Pain Med, 2008. **9**(8): p. 979-84.

15. Mouyis, M., et al., *Hypovitaminosis D among rheumatology outpatients in clinical practice.* Rheumatology (Oxford), 2008. **47**(9): p. 1348-51.

16. Al-Allaf, A.W., et al., *Bone health in patients with fibromyalgia.* Rheumatology (Oxford), 2003. **42**(10): p. 1202-6.

17. Ahmed, W., et al., *Low serum 25 (OH) vitamin D levels (<32 ng/mL) are associated with reversible myositis-myalgia in statin-treated patients.* Transl Res, 2009. **153**(1): p. 11-6.

18. de Torrente de la Jara, G., A. Pecoud, and B. Favrat, *Female asylum seekers with musculoskeletal pain: the importance of diagnosis and treatment of hypovitaminosis D.* BMC Fam Pract, 2006. **7**: p. 4.

19. Kragstrup, T.W., *Vitamin D supplementation for patients with chronic pain.* Scand J Prim Health Care, 2010.

20. Arnson, Y., D. Amital, and H. Amital, *The diverse world of vitamin D: does it also modulate pain sensation?* Isr Med Assoc J, 2009. **11**(6): p. 371-2.

21. de Rezende Pena, C., L.P. Grillo, and M.M. das Chagas Medeiros, *Evaluation of 25-hydroxyvitamin d serum levels in patients with fibromyalgia.* J Clin Rheumatol, 2010. **16**(8): p. 365-9.

22. Tandeter, H., et al., *Serum 25-OH vitamin D levels in patients with fibromyalgia.* Isr Med Assoc J, 2009. **11**(6): p. 339-42.

23. Straube, S., et al., *Vitamin D and chronic pain.* Pain, 2009. **141**(1-2): p. 10-3.

24. Straube, S., et al., *Vitamin d and chronic pain in immigrant and ethnic minority patients-investigation of the relationship and comparison with native Western populations.* Int J Endocrinol, 2010. **2010**: p. 753075.

25. Myers, K.J., *Vitamin D deficiency and chronic pain: cause and effect or epiphenomenon?* Mayo Clin Proc, 2004. **79**(5): p. 695; author reply 695-6.

26. Block, S.R., *Vitamin D deficiency is not associated with nonspecific musculoskeletal pain syndromes including fibromyalgia.* Mayo Clin Proc, 2004. **79**(12): p. 1585-6; author reply 1586-7.

27. Straube, S., et al., *Vitamin D for the treatment of chronic painful conditions in adults.* Cochrane Database Syst Rev, 2010(1): p. CD007771.

28. Warner, A.E. and S.A. Arnspiger, *Diffuse musculoskeletal pain is not associated with low vitamin D levels or improved by treatment with vitamin D.* J Clin Rheumatol, 2008. **14**(1): p. 12-6.

29. Branco, J.C., *State-of-the-art on fibromyalgia mechanism.* Acta Reumatol Port, 2010. **35**(1): p. 10-5.

30. Staud, R., *Biology and therapy of fibromyalgia: pain in fibromyalgia syndrome.* Arthritis Res Ther, 2006. **8**(3): p. 208.

31. Staud, R., *Is it all central sensitization? Role of peripheral tissue nociception in chronic musculoskeletal pain.* Curr Rheumatol Rep, 2010. **12**(6): p. 448-54.

32. Bradley, L.A., *Pathophysiology of fibromyalgia.* Am J Med, 2009. **122**(12 Suppl): p. S22-30.

33. Solitar, B.M., *Fibromyalgia: knowns, unknowns, and current treatment.* Bull NYU Hosp Jt Dis, 2010. **68**(3): p. 157-61.

34. Staud, R., et al., *Enhanced central pain processing of fibromyalgia patients is maintained by muscle afferent input: a randomized, double-blind, placebo-controlled study.* Pain, 2009. **145**(1-2): p. 96-104.

35. Watkins, L.R., E.D. Milligan, and S.F. Maier, *Spinal cord glia: new players in pain.* Pain, 2001. **93**(3): p. 201-5.

36. Ceglia, L., *Vitamin D and skeletal muscle tissue and function.* Mol Aspects Med, 2008. **29**(6): p. 407-14.

37. Pfeifer, M., B. Begerow, and H.W. Minne, *Vitamin D and muscle function.* Osteoporos Int, 2002. **13**(3): p. 187-94.

38. Bischoff, H.A., et al., *Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial.* J Bone Miner Res, 2003. **18**(2): p. 343-51.

39. Bischoff-Ferrari, H.A., et al., *Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y.* Am J Clin Nutr, 2004. **80**(3): p. 752-8.

40. Pfeifer, M., et al., *Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women.* J Bone Miner Res, 2000. **15**(6): p. 1113-8.

41. Pfeifer, M., et al., *Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals.* Osteoporos Int, 2009. **20**(2): p. 315-22.

42. Wicherts, I.S., et al., *Vitamin D status predicts physical performance and its decline in older persons.* J Clin Endocrinol Metab, 2007. **92**(6): p. 2058-65.

43. Kiraly, S.J., et al., *Vitamin D as a neuroactive substance: review.* ScientificWorldJournal, 2006. **6**: p. 125-39.

44. Mehta, N.M., A. Malootian, and J.P. Gilligan, *Calcitonin for osteoporosis and bone pain.* Curr Pharm Des, 2003. **9**(32): p. 2659-76.

45. Eyles, D.W., et al., *Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain.* J Chem Neuroanat, 2005. **29**(1): p. 21-30.

46. Roesel, T.R., *Does the central nervous system play a role in vitamin D deficiency-related chronic pain?* Pain, 2009. **143**(1-2): p. 159-60.

47. Armstrong, D.J., et al., *Vitamin D deficiency is associated with anxiety and depression in fibromyalgia.* Clin Rheumatol, 2007. **26**(4): p. 551-4.

48. Rosado Mda, L., et al., *[Cultural adaptation and validation of the "Fibromyalgia Impact Questionnaire"--Portuguese version].* Acta Reumatol Port, 2006. **31**(2): p. 157-65.

49. Bennett, R., *The Fibromyalgia Impact Questionnaire (FIQ): a review of its development, current version, operating characteristics and uses.* Clin Exp Rheumatol, 2005. **23**(5 Suppl 39): p. S154-62.

50. Chapuy, M.C., et al., *Prevalence of vitamin D insufficiency in an adult normal population.* Osteoporos Int, 1997. **7**(5): p. 439-43.

51. Holick, M.F., *Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis.* Am J Clin Nutr, 2004. **79**(3): p. 362-71.

Appendix

Appendix 1 – Socio-demographic Interview

INQUÉRITO DEMOGRÁFICO

Data: ____/____/_____

Nº: 00 A

Iniciais do nome (1,2 e última): _____

Data de nascimento: _____/___/___

Nacionalidade: _____

Residiu no estrangeiro até aos 18 anos? _____

Desde que idade: _____ anos. Regressou aos: _____ anos.

Número de irmãos: _____

Posição na fratria: _____ (do mais velho para o mais novo)

Sexo dos irmãos (F-feminino, M-masculino): ___; ___; ___; ___; ___; ___; ___; ___

(do mais velho para o mais novo)

Tem filhos? Sim ☐ Não ☐

Nº total de filhos vivos: ____ Abortos espontâneos: _____ Abortos Provocados: ____

Filhos falecidos: ____

1.: idade à data da morte: ____ Ano da morte: _____

2.: idade à data da morte: ____ Ano da morte: _____

3.: idade à data da morte: ____ Ano da morte: _____

Pai: vivo ☐ separado ☐ Ano: _____ Falecido ☐ Ano: _____

Estado civil: _____

Profissão:

Por conta própria ☐ Por conta de outrem ☐

Desempregada ☐ Reformada ☐ Com que idade? _____ anos

Anos de educação formal: _____ Concluídos em: ____ anos

Critérios de Fibromialgia: Sim ☐ Não ☐

Data de início dos sintomas (mês e ano): ____/_____

Data em que primeiro procurou cuidados médicos por esses sintomas (mês e ano): ____/_____

Data do diagnostico (mês e ano): ____/_____

Que tratamentos faz actualmente para a Fibromialgia? (fármacos e outros)

_____
_____
_____
_____
_____
_____

Se tem outras doenças, indique quais:

_____
_____
_____
_____

Que tratamentos faz para estas doenças?

_____
_____
_____
_____

Se já fez cirurgias, indique quais, e o ano da cirurgia:

_____
_____
_____
_____

Quem foi, para si, a principal figura maternal durante a sua infância, até aos sete anos?

_____

Appendix 2 – Physical Examination

EXAME FÍSICO

Peso: _____ kg Altura: _____ m IMC: _____ kg/m^2

Nº de pontos dolorosos: ____

Limiar de dor:		**A**	**B**	**Média**
a. Ponto médio da tíbia:	Dta:	_____Kg	_____Kg	_____Kg
	Esq:	_____Kg	_____Kg	_____Kg
b. Ponto médio do manúbrio esternal:		_____Kg	_____Kg	_____Kg
c. Leito ungueal do indicador:	Dta:	_____Kg	_____Kg	_____Kg
	Esq:	_____Kg	_____Kg	_____Kg

Massa gorda:

a. Perímetro abdominal: _____ cm
(entre o umbigo e a linha que passa nas cristas ilíacas, numa expiração normal)

b. Prega bicipital:
(medida três vezes no braço não dominante)

A	**B**	**C**	**Média**
_____cm	_____cm	_____ cm	_____ cm

c. Prega tricipital:
(medida três vezes no braço não dominante)

A	**B**	**C**	**Média**
_____cm	_____cm	_____ cm	_____ cm

Appendix 3 – Fibromyalgia Impact Questionnaire (Versão Portuguesa) – FIQ-P

FIBROMYALGIA IMPACT QUESTIONNAIRE
(VERSÃO PORTUGUESA) – FIQ-P

INSTRUÇÕES: Nas perguntas 1 a 11 por favor faça um círculo no número que, em relação à **última semana**, melhor descreve a maneira como, **em geral**, foi capaz de executar as tarefas indicadas. Se habitualmente não faz uma dessas tarefas risque essa pergunta.

	Sempre	Quase Sempre	Quase nunca	Nunca
Foi capaz de:				
1. Ir às compras?	0	1	2	3
2. Tratar da roupa na máquina de lavar / secar?	0	1	2	3
3. Cozinhar?	0	1	2	3
4. Lavar louça à mão?	0	1	2	3
5. Aspirar a casa?	0	1	2	3
6. Fazer as camas?	0	1	2	3
7. Andar vários quarteirões (200 a 500 metros)?	0	1	2	3
8. Visitar a família ou os amigos?	0	1	2	3
9. Tratar das plantas ou praticar o seu passatempo?	0	1	2	3
10. Se deslocar, no seu próprio carro ou em transportes públicos?	0	1	2	3
11. Subir as escadas?	0	1	2	3

12. Na última semana, em quantos dias se sentiu bem?

0	1	2	3	4	5	6	7

13. Na última semana, quantos dias faltou ao trabalho e/ou não realizou as tarefas domésticas, devido à fibromialgia?

0	1	2	3	4	5	6	7

INSTRUÇÕES: Nas perguntas que se seguem, assinale um ponto na linha que melhor indica o modo como, **em geral**, se sentiu na **última semana**.

14. Nos dias que trabalhou, quanto é que a sua doença – Fibromialgia - interferiu no seu trabalho?

Trabalhei sem problemas •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Tive grande dificuldade no trabalho

15. Que intensidade teve a sua dor?

Não tive dor •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Tive dor muito intensa

16. Que cansaço sentiu?

Não senti cansaço •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Senti um cansaço enorme

17. Como se sentiu quando se levantava de manhã?

Acordei bem repousada •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Acordei muito cansada

18. Que rigidez sentiu?

Não tive rigidez •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Senti muita rigidez

19. Sentiu-se nervosa ou ansiosa?

Não tive ansiedade •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Senti-me muito ansiosa

20. Sentiu-se triste ou deprimida?

Não me senti deprimida •___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |___• Senti-me muito deprimida

Appendix 4 – Statistical Analysis

Paired Samples t-test and Paired Samples Correlations between vitamin D levels in FM patients and their sisters

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Vitamin D FM group - Vitamin D Control Group	2,91667	7,96428	1,73795	-,70863	6,54197	1,678	20	,109

		N	Correlation	Sig.
Pair 1	Vitamin D FM group & Vitamin D Control Group	21	,061	,794