Methylation of the NR3C1 gene in individual with chronic pain: patient profile in a cross-sectional study with users of the public health system

Metilação do gene NR3C1 em indivíduos com dor crônica: perfil de pacientes em estudo transversal com usuários do sistema público de saúde

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ABSTRACT

BACKGROUND AND OBJECTIVES: Studies suggest that shared molecular factors can simultaneously affect different chronic pain syndromes. Understanding the epigenetic mechanisms of various diseases that are associated with chronic pain is essential to comprehend its appearance and progression. The objective of this study is to evaluate the association between DNA methylation of the NR3C1 gene with the presence and intensity of chronic pain, as well as predictive factors also considering socioeconomic, health and lifestyle factors correlated with this association, in adult individuals using the Brazilian Unified Health System (*Sistema Único de Saúde* - SUS) in a municipality in Southeast Brazil.

METHODS: This is a cross-sectional study, whose data collection was carried out through interviews to investigate socioeconomic status, lifestyle and health conditions, in addition to anthropometric assessments and blood samples. Data were analyzed by quantitative DNA methylation assays and statistical analysis.

CONCLUSION: The findings suggest epigenetic involvement in *NR3C1* gene methylation in association with chronic pain and suggest the need to seek new evidence in relation to the mechanisms that explain chronic pain, especially from an epigenetic point of view, as they may provide subsidies for the prevention and control of chronic pain targeting individuals with the profile found in this study.

Keywords: Chronic pain, DNA methylation, Life style, Socioeconomic factors.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Estudos sugerem que fatores moleculares compartilhados podem afetar simultaneamente diferentes síndromes de dor crônica. Compreender os mecanismos epigenéticos de várias doenças que estão associadas à dor crônica é essencial para entender sua aparência e progressão. O objetivo deste estudo foi avaliar a associação entre metilação do DNA do gene *NR3C1,* receptor de glicocorticoides, com a presença e intensidade de dor crônica, bem como os fatores preditivos considerando também fatores socioeconômicos, de saúde e de estilo de vida correlacionados com tal associação em pacientes adultos usuários do Sistema Único de Saúde (SUS) em um município do sudeste brasileiro.

MÉTODOS: Trata-se de um estudo observacional transversal, cuja coleta de dados foi realizada através de entrevistas para investigação do *status* socioeconômico, condições de estilo de vida e de saúde, além de avaliações antropométricas e coletas de sangue. Os dados foram analisados por meio de ensaios quantitativos de metilação do DNA e análise estatística.

RESULTADOS: Foi observado que 123 participantes (44,1%) apresentaram metilação da região estudada do gene *NR3C1*. Análises estatísticas univariadas e multivariada mostraram que as variáveis idade e nível de cortisol estão significativamente associadas com a metilação do gene e a presença de dor crônica.

CONCLUSÃO: Os achados sugerem envolvimento epigenético na metilação do gene *NR3C1* em associação com dor crônica e sugerem a necessidade de se buscar novas evidências em relação aos mecanismos que explicam a dor crônica, sobretudo do ponto de vista epigenético, pois as mesmas poderão trazer subsídios para prevenção e controle da dor crônica visando pacientes com o perfil encontrado nesse estudo.

Descritores: Dor crônica, DNA metilação, Estilo de vida, Fatores socioeconômicos.

INTRODUCTION

According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential

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HIGHLIGHTS

- Suggests epigenetic involvement in association with chronic pain.
- Identifies the profile of individuals affected by chronic pain.
- Evidence subsidies for the prevention and control of chronic pain.

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tissue damage[1]. Regarding its duration, pain can be classified as acute (appearing suddenly, punctually, resulting from trauma or associated to diseases, lasting less than six months) or chronic (persisting over time, lasting more than six months). Chronic pain affects a large part of the world's population, both in developed and developing countries. Research has examined the relationship of chronic pain and stress, a relatively important psychobiological process[1,2].

When an individual is exposed to situations that lead to stress, a molecular cascade initiated by the hypothalamus happens in the hypothalamic-pituitary-adrenal (HPA) axis, with secretion of corticotrophin-releasing hormone (CRH), which, in turn, causes the release of adrenocorticotropic hormone (ACTH) in the pituitary gland, resulting in the production of glucocorticoids in the adrenal cortex. Glucocorticoid receptors switch off this stress response through negative feedback, which is impaired under stressful situations[3].

Epigenetics is an emerging area of research that studies how environmental influences can affect the expression of an individual's genes. Epigenetic processes such as histone acetylation and methylation may be involved in the pathogenesis of chronic pain (CP). Studies suggest that shared molecular factors may simultaneously affect different CP syndromes. Comprehending the epigenetic mechanisms of various diseases that are associated with CP is essential to understanding the appearance and progression of the disease. Efforts have been made in discovering genes that may be responsible for the onset of pain, however, it is important to consider not only individual pain-related genes, but also to take a more holistic approach that considers epigenetic mechanisms[4,5].

There are reports in the literature of an association between CP and alterations in gene expression and regulation. Thus, pain can epigenetically alter genes such as SCN9A, ZFHX2, 5HT2A, COMT[6-8].

The NR3C1 gene, also called glucocorticoid receptor gene, or simply GR, encodes the human glucocorticoid receptor, which is a ligand-dependent transcription factor and activates transcription of glucocorticoid responsive genes through direct binding to glucocorticoid response elements in its promoter region, or by modulating the transcriptional activity of other transcription factors through protein-protein interactions. It is implicated in a broad spectrum of physiological and biochemical functions that are essential for life and play a key role in maintaining basal and stress-related homeostasis. Increased methylation impairs plasticity in NR3C1 gene expression and, consequently, the range of future stress adaptation responses, possibly resulting in increased risk for chronic diseases of onset in adulthood[9].

The aim of this study was to evaluate the predictive factors and the association between DNA methylation of the NR3C1 gene with the presence and intensity of CP, considering also socioeconomic, health, and lifestyle factors in adult individuals.

METHODS

A cross-sectional observational study, following the recommendations of the STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology)[10], consisting of 386 patients recruited from the *Sistema Unificado de Saúde* (SUS - Brazilian Unified Health System) in the municipality of Alegre-ES, southeastern Brazil. Of this total, 279 patients were selected based on the following eligibility criteria for participation in the study: ages between 20 and 59 years, being the only individual residing at their home to participate in the research, and having cognitive conditions to answer the questionnaires.

The individuals were invited by the researchers to participate in the research through home visits, accompanied by the community health agent in urban and rural localities of the municipality in the period between 2014 and 2016.

Variables and measurement instruments

Independent variables were studied through individual interviews. Subsequently, patients were categorized regarding gender, age, per capita income (Getúlio Vargas Foundation, 2014), schooling, having or not having children, report of comorbidities, self-reported stress and anxiety, self-reported health, food and nutritional security, overweight, alcohol consumption, smoking habit, self-reported use of continuous drugs (for depression, sleep, blood pressure, diabetes, heart disease), practice of physical activity and alcohol consumption. The dependent variable was pain intensity, and the Brief Pain Inventory (BPI) scores between 1 and 3.9 were considered for mild pain; between 4 and 6.9 for moderate pain; and between 7 and 10 for severe pain[11].

The anthropometric evaluation was performed between 7:00 and 8:00 a.m., with the participants fasting for at least 8 hours. Height was measured with a stadiometer (Alturexata®, Belo Horizonte, MG, Brazil) and body weight was measured with an electronic scale (Tanita®, model BC601, São Bernardo do Campo, SP, Brazil). Body mass index (BMI) was calculated and classified following the reference for adults (20-59 years old)[12].

Segurança Alimentar e Nutricional (SAN - Food and Nutrition Security) was assessed using the *Escala Brasileira de Insegurança Alimentar* (EBIA - Brazilian Scale of Food Insecurity)[13-15]. The EBIA is composed of 14 questions with a final score resulting from the sum of affirmative answers. Based on the sum, data are provided on the degree of food insecurity (mild, moderate, and severe), referring to the concern about lack of food in the home, and the fact that a resident has been hungry for a period of one day in the last three months. Responses were classified as "YES" among those with food security, that is, *segurança alimentar* or SAN (EBIA=0) and "NO" among those with mild, moderate or severe food insecurity, or *insegurança alimentar* INSAN (EBIA>0).

Assessment of chronic pain

Symptoms suggesting CP were investigated using the BPI, whose values obtained were appropriate to the categorized total scores. The BPI evaluates the presence of pain in any part of the body and pain intensity. To evaluate pain intensity, the BPI scores between 1 and 3.9 were considered for mild pain; between 4 and 6.9 for moderate pain; and between 7 and 10 for severe pain[11].

Depression assessment

Symptoms suggesting depression were investigated by means of the Beck Depression Inventory-II - BDI-II, a self-report scale capable of assessing the presence and intensity of depressive symptoms, widely used in clinical practice and in research with the general popula-

tion[16]. The BDI-II consists of a questionnaire containing 21 items that assess the person's feelings over the past two weeks, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), classified on a 4-point scale, ranging from 0 to 3, with a maximum possible score of 63 points[17]. From each patient's score, the outcome variable studied was determined as "depressive symptoms", with positive screening defined by a BDI-II ≥ 17[18] score.

Blood samples, cortisol dosage, and DNA extraction

Samples of 10 mL of peripheral blood were collected through venipuncture, transported in thermal boxes to the Biotechnology Laboratory at UFES for processing. 3 mL of the sample was transferred to a tube with EDTA (ethylenediaminetetraacetic acid) anticoagulant for molecular analysis and 2 mL to a tube containing NaF (sodium fluoride) anticoagulant. The remaining sample was transferred to a tube without anticoagulant, containing a separator gel for the determination of cortisol levels. Serum cortisol analysis was performed by the chemiluminescence method (reference values: 6.7 to 22.6 µg/dL), categorizing cortisol as low: < 6.7 µg/dL higher than normal cortisol: ≥6.7 µg/dL (Laboratório Hermes Pardini®, Belo Horizonte, MG, Brazil). DNA extraction was performed by the Salting-Out method with saline precipitation[19]. DNA quality and concentration was checked using NanoDrop® (São Paulo, SP, Brazil).

Quantitative assays of DNA methylation

From the extracted DNA samples, the sodium bisulfite conversion was performed using a kit (EpiTect® Bisulfite Kit; Qiagen, Valencia, CA), following the manufacturer's recommendations. Confirmation of PCR product quality and absence of contamination was established on 2% agarose gels using GelRedTM® (Uniscience, Osasco, SP, Brazil).

The 1F region of NR3C1 contains 410 base pairs and 47 CpGs sites[20,21]. Primers including the region between 988 and 1344 (sequence data submitted to the GenBank database with NCBI access number AY436590.1) were used[22]. The NR3C1 sequence, region 1F, which encompasses CpGs 40 to 47, was analyzed[23].

Pyrosequencing was performed using the PSQ96ID Pyrosequencer® (Qiagen, Valencia, CA) with the PyroMark Gold Q96 Reagent Kit® (Qiagen, Valencia, CA), according to the manufacturer's protocol.

Bias

All interviews and data collection were performed using standardized instruments, collected by the researchers themselves, as well as the collection of material for cortisol and DNA analysis. The assessment instruments were used according to specific recommendations, and the blood samples were collected, stored, transported, and analyzed according to the described standardization.

Sample size

The sampling was based on the e-SUS registry of the population aged 20 to 59 years, living in Alegre, ES, and served by the *Rede de Atenção Básica* (Primary Care Network), which was previously provided by the *Agentes Comunitários de Saúde* (CHAs - Community Health Agents) linked to the *Programa de Agentes Comunitários de Saúde* (PACS - Community Health Agents Program) and *Unidades Básicas de Saúde* (UBS – Basic Health Care Units) in the city.

For the sample calculation the formula presented below was used through the open software OpenEpi, version 3.01, assuming an absolute precision of 5%, confidence interval of 95%, design effect equal to 1 and, in the absence of specific studies in the region, the estimated overweight proportion of 50% was taken as reference. Finally, 10% losses were added. The sample was stratified proportionally by rural and urban location, as well as by the UBS and PACS, with the objective of representing the *Rede de Atenção Básica Municipal* (Municipal Primary Care Network).

$$n = deff \times \frac{N\hat{p}\hat{q}}{\frac{d^2}{1.96^2}(N-1) + \hat{p}\hat{q}}$$

n = sample size; N = population; d = desired absolute precision; p = estimated proportion of the event; q = 1 - p; deff = design effect.

Source: Schaeffer & Mendenhall (1990)

The present study was approved by the Ethics Committee on Research with Human Beings of the Health Sciences Center of the Federal University of Espírito Santo (CEP/CCS/UFES) on June 6, 2016 under CAAE number 52830216.5.0000.5060, approved under Opinion number 1.574.160. All participants signed the Free Informed Consent Term (FICF).

Statistical analysis

A descriptive analysis characterized the sample, starting with the presentation of the variables as means, standard deviations, frequencies, and proportions. The Kolmogorov-Smirnov normality test evaluated the normality of the quantitative variables. For the Poisson regression analysis, the pain intensity variable was categorized dichotomously into mild and moderate/intense. The result of percentage of the mean methylation values of CpGs 40-47 of the NR3C1 gene region recategorized dichotomously were considered as unmethylated (0%) and methylated (>0%).

The Poisson regression analysis with robust variance was used to identify the potential predictive factors of CP. First, the univariate analysis was performed and the variables with p-value ≤ 0.20 were selected to compose the multivariate model. Next, the predictive variables were inserted into the multivariate model, adopting the Backward modeling strategy, in which the variables with the highest p value were removed one by one, until the final reduced model was reached, in which all remaining variables presented p ≤ 0.05. The Hosmer & Lemeshow test was used to verify the adjustment of the final model.

Statistical analyses were performed using the software Stata®, version 14.1 (StataCorp® LP, College Station, Texas, USA) and SPSS®, version 20.0 (IBM, Munich, Bavaria, Germany).

RESULTS

Considering the selected and studied indicators, the authors observed that most of the participants presented CP and,

Table 1. Sample characterization

Factors	% (n)	p-value	IRR (95% CI)
BPI ≥ 4	63.7 (123)		
Gender			
Female	79.9 (223)	1	
Male	20.1 (56)	≤ 0.296	1.25 (0.82 – 1.90)
Age			
20 to 40 years old	42.6 (119)	1	
41 to 59 years old	57.4 (160)	≤ 0.001*	1.03 (1.01 – 1.05)
Per capita income			
Low income	41.2 (115)	1	
Not low income	58.8 (164)	≤ 0.015*	0.77 (0.63 – 0.95)
Schooling			
< 9 years of study	82.1 (229)	1	
> 9 years of study	17.9 (50)	≤ 0.007*	0.64 (0.46 – 0.89)
Children			
Yes	79.9 (223)	1	
No	20.1 (56)	≤ 0.028*	1.76 (1.06 – 2.91)
Report of comorbidities			
No	66.7 (186)	1	
Yes	33.3 (93)	≤ 0.005*	1.54 (1.14 – 2.10)
Self-reported stress and anxiety			
No	33.7 (94)	1	
Yes	66.3 (185)	≤ 0.463	1.15 (0.79 – 1.70)
How do you evaluate your health			
Satisfactory	54.5 (152)	1	
Unsatisfactory	45.5 (127)	≤ 0.051*	1.37 (1.00 – 1.87)
Food and nutrition security			
No	45.2 (126)	1	
Yes	54.8 (153)	≤ 0.011*	1.50 (1.10 – 2.04)
Overweight			
No	35.1 (98)	1	
Yes	64.9 (181)	≤ 0.141*	1.01 (0.99 – 1.04)
Weekly alcohol consumption			
Less than two doses	82.1 (229)	1	
More than two doses	17.9 (50)	≤ 0.071*	1.85 (0.95 – 3.63)
Currently smoking			
No	90.1 (253)	1	
Yes	9.9 (26)	≤ 0.124*	1.40 (0.91 – 2.17)
Continuous drug use			
No	45.9 (128)	1	
Yes	54.1 (151)	≤ 0.001*	1.39 (1.16 – 1.66)
Practice of physical activities			
No	62 (173)	1	
Yes	38 (106)	≤ 0.002*	1.30 (1.10 – 1.53)
Low cortisol levels			
No	88.9 (248)	1	2.26e-09
Yes	11.1 (31)	≤ 0.001*	(1.00e-09 – 5.12e-09)
Depression			
No	84.2 (235)	1	
Yes	15.8 (44)	≤ 0.437	1.15 (0.81 – 1.63)
CpG42 methylation			
No	60.2 (230)	1	
Yes	39.8 (49)	≤ 0.001*	0.00 (0.00 – 0.01)

BPI = Brief Pain Inventory; IRR = incidence ratio; CI = confidence interval.

of these, most reported pain of moderate/severe intensity. Among patients with pain, most participants were women, over 40 years old, average per capita income, lower schooling, with children, without reports of one or more comorbidities, with self-reported stress and anxiety, assessed their health as satisfactory, presented food and nutritional security, were overweight, consumed less than two doses of alcoholic beverages a week, did not smoke, used continuous drugs, did not practice physical activity, and presented normal levels of cortisol, as seen in table 1.

It was observed that 123 participants (44.1%) had methylation of the NR3C1 gene studied region, as seen in table 2 and figure 1, which presents methylation data in the sample of individuals with CP, in addition to the methylation data of each CpG area of the gene.

Table 2. Prevalence of the NR3C1 gene methylation in the sample of individuals with chronic pain (total and by CpG)

Methylation Data	Result % (n)
Total methylation	
No	55.9% (156)
Yes	44.1% (123)
Methylation by CpG site	
CpG40	20.3% (25)
CpG41	2.5% (3)
CpG42	39.8% (49)
CpG43	2.5% (3)
CpG44	6.5% (8)
CpG45	5.7% (7)
CpG46	18.6% (23)
CpG47	4.1% (5)

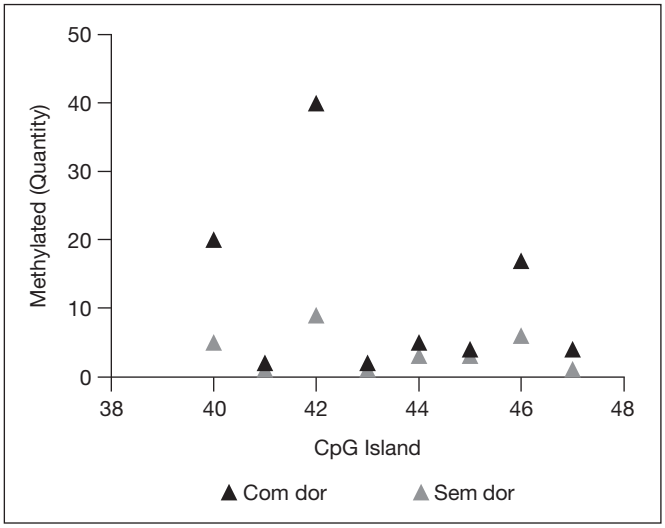

Figure 1. Association between methylation versus pain by CpGc island.

The analysis through univariate Poisson regression resulted in the selection of the variables that presented a value of p ≤ 0.200, used to compose the following multivariate model, by the Backward method, as seen in tables 3 and 4.

Table 3. Variables associated with moderate/severe pain intensity by the univariate Poisson regression analysis with robust variance

Independent variables	IRR	CI		p-value
Gender	1.25	0.82	1.90	≤ 0.296
Age	1.03	1.01	1.05	≤ 0.001*
Per capita income	0.77	0.63	0.95	≤ 0.015*
Schooling	0.64	0.46	0.89	≤ 0.007*
Having children	1.76	1.06	2.91	≤ 0.028*
Report of comorbidities	1.54	1.14	2.10	≤ 0.005*
Self-reported stress and anxiety	1.15	0.79	1.70	≤ 0.463
How do you rate your health	1.37	1.00	1.87	≤ 0.051*
Food and nutrition security	1.50	1.10	2.04	≤ 0.011*
Overweight	1.01	0.99	1.04	≤ 0.141*
Weekly alcohol consumption	1.85	0.95	3.63	≤ 0.071*
Currently smoking	1.40	0.91	2.17	≤ 0.124*
Continuous drug use	1.39	1.16	1.66	≤ 0.001*
Practice of physical activities	1.30	1.10	1.53	≤ 0.002*
High cortisol levels	2.26e-09	1.00e-09	5.12e-09	≤ 0.001*
Depression	1.15	0.81	1.63	≤ 0.437
CpG42	0.00	0.00	0.01	≤ 0.001*

IRR = incidence ratio; CI = confidence interval.

Table 4. Variables associated with moderate/severe pain intensity by multivariate Poisson regression analysis with robust variance (final reduced model).

Independent variables	IRR	IC		p-value
Age	1.03	1.01	1.05	≤ 0.001
High cortisol levels	4.64e-10	1.95e-10	1.10e-09	≤ 0.001
CpG42 methylation	0.02	0.01	0.02	≤ 0.001

IRR = incidence ratio; CI = confidence interval.

DISCUSSION

The present study observed that approximately 70% of the included participants have CP, and approximately 64% of these had moderate/intense CP. Genetic factors play an important role in the etiology of CP conditions, presumably by modulating underlying processes such as nociceptive sensitivity, psychological well-being, inflammation, and autonomic response, and may represent an important risk marker for pain and identify potential targets for therapeutic intervention[24].

An epidemiological study conducted by the *Sociedade Brasileira para o Estudo da Dor* (SBED - Brazilian Society for the Study of Pain) in 2015 showed that the incidence of CP in Brazil ranges from 28 to 42% in the different areas of the country. Epidemiological data on CP in the world point to evidence that it has a detrimental effect on physical health, activities of daily living, mental health, economic conditions, and well-being[25].

Pain is a stressful situation; therefore, it is configured as a potential mechanism that can alter stress regulation. In this context, part of the attention of researchers is directed to the HPA axis. Although it is not directly involved in pain management, it is known that the CRH-producing neurons innervate areas located in the arcuate nucleus of the hypothalamus and in areas that control pain in the spinal cord and in the hindbrain (bridge, cerebellum, and medulla). Activation of the stress response system causes secretion of CRH-induced peptides as well as other opioid peptides, which increases analgesia[26].

Epigenetic molecular mechanisms including DNA methylation are implicated in modulating the HPA axis. The NR3C1 gene methylation can increase due to stressful situations[20,23,27] and thus may be involved in chronic stress-induced pain. Further investigation of the epigenetic involvement of the HPA axis genes in pain may provide a basis to explore the epigenetic mechanisms that may contribute to the similar symptomatology in several diseases that have pain as one of the symptoms[28].

Research on epigenetics also investigates possible biomarkers for CP and focuses on studying the epigenome, histone acetylation, and other genes not related to stress[29-31]. Few studies focus on the evaluation of methylation in genes of the HPA axis in relation to pain, specifically the NR3C1 gene. The authors[32] showed that chronic stress alters HPA axis regulation and increases pain perception in rats due to the increase of NR3C1 methylation in the axis 1 promoter region in nociceptive neurons innervating the pelvic viscera. In addition, NR3C1 mRNA and GR protein were decreased. Therefore, the authors support the interpretation that epigenetic regulatory mechanisms play a central role in chronic stress-induced visceral hyperalgesia, affecting nociceptive neurons in a region-specific manner.

Similarly, the study[33] review exposes the association between environmental exposure, chronic stress, and epigenetic changes. The authors mention that chronic stress can lead to changes in DNA methylation, including of the NR3C1 gene, which plays an essential role in the epigenetic modulation of CP, contributing to its chronicity and intensity.

In addition, patients with fibromyalgia showed alterations in NR3C1 methylation and lower mRNA expression of GR and MR (mineralocorticoid receptor), and lower cortisol levels associated with impaired function in the HPA axis[34].

Contrary to expectations, an inverse relationship was found between NR3C1 methylation and moderate/intense CP. This result may be explained by the fact that prolonged stress caused by CP may have led to a state of low stress responsiveness, as an indicator of chronic stress, possibly related to a blunted response of the HPA axis[35].

DNA methylation in the promoter region of NR3C1 may indicate reduced gene transcription and GR expression in the central nervous system and is strongly associated with the imbalance of HPA axis modulation and consequently may indicate alterations in responsiveness to stress[21,36]. Previous stu-

dies have focused on NR3C1 methylation in association with chronic stress, such as in prenatal adversity[20,37], childhood adversity[21,23,38], post-traumatic stress disorder[36,39], suicide[40], depression[41,42] and other situations of prolonged stress[41].

This data is indeed confirmed by the multivariate regression result, in which high cortisol also presented an inverse association with moderate/intense pain, considering the significance of an inverse relationship (the higher the cortisol level, the lower the pain intensity), therefore, the lower the cortisol levels, the higher the pain intensity.

According to the *Sociedade Brasileira de Endocrinologia e Metabologia* (SBEM - Brazilian Society of Endocrinology and Metabology), cortisol is a corticosteroid produced by the adrenal glands, and the reduction of its production due to adrenal insufficiency may trigger symptoms such as hypotension, chronic fatigue, muscle weakness, muscle and joint pain, dizziness, headache, nausea, vomiting, diarrhea, loss of appetite, weight loss, darkening of the skin and lips[43].

In the literature, studies show controversial data in highlighting which cortisol level is predominant in individuals with CP. Most studies suggest or show a tendency toward hypocortisolism, but others show no difference, and still others suggest hypercortisolism. Segments of patients who had a worsening of CP in a period longer than one year show low cortisol levels with the evolution of pain, and studies in women with fibromyalgia show an association of cortisol levels and pain and depression symptoms, which points to a relationship between the daily variation of cortisol release with the intensity of the painful sensation[26,44].

Low cortisol levels upon awakening and cortisol decline throughout the day have been considered predictors of fatigue, burnout, and life exhaustion. Professionals who work long shifts are especially susceptible to chronic stress and fatigue, whose long-term consequences include the desynchronization of the HPA axis and consequent physical and mental illness, decreased work capacity, increased occupational accidents, and absenteeism[45].

Pain intensity may vary according to triggering factors, with prevalent rates in patients with accumulated tasks and demands that generate impacting daily stress, showing multiple associated factors such as anxiety, depression and emotional suffering, history of abuse (physical, psychological and sexual) and abandonment, conditions related to post-traumatic stress with low immunity, inflammation and infection recurrence, nerve compression, chronic musculoskeletal tension and history of occupational bad posture[46].

In the present study, among the patients that reported moderate/severe CP, the majority are older than 40 years. It is estimated that the higher the age, especially in the seniors (2/3), the more cases of those who suffer frequent or permanent pain in the later years of their existence, and that the proportion of pain increases by up to 26% with age. The population of longevous people has increased, and the aging population is often a social problem directly related to public health programs. Aging is part of the life cycle, and is therefore inevitable. It is a biological process in which alterations determine structural and functional changes in the human body, leading to a decrease in some physiological functions, also slowing down reflexes, making some seniors disabled due to the frequency of chronic diseases, which need to be treated with drugs of continuous use, and many of them can be expensive[47,48].

Despite progress in treatments, pain is still insufficiently treated, especially in older age. Diseases prone to cause pain increase with age, such as rheumatism, arthrosis, and cancer, among others. Older patients also face communication and memory problems and are unable to express their pain clearly. In addition, many find it normal to feel pain in old age and do not seek help[25].

Therefore, the comprehension of epigenetic mechanisms in several diseases associated with CP becomes essential to understand it, also taking into account a holistic approach to pain[3,5].

It is worth noting that, in this study, although the self-reported stress variable did not compose the final reduced model, it was present in 66.3% of the participants. Thus, the associations between pain, behavioral and physical aspects, and quality of life in the aging population need evidence to prevent and treat pain in this age group, verifying the relationship between the levels of chronic pain and stress levels[49] more efficiently.

The link between CP and affective components, such as depression and anxiety, has been established in the literature over the years, with studies reporting the presence of pain in depression, anxiety, and coexistence of both in CP. Among psychiatric disorders, major depression has been more extensively studied and its occurrence has been better established in patients with CP. Prevalence studies of psychiatric comorbidities associated with CP refer firstly to mood disorders, among which depressive disorders reach percentages between 30% and 87% of cases. Among the anxiety disorders, which reach 50% of the cases, the most frequent are panic disorder, generalized anxiety disorder, and post-traumatic stress disorder. As a symptom, anxiety is present in 56% of the cases[42].

CP affects the patients' physical, psychological, and social aspects, in addition to generating damages in different areas of their lives, resulting in worries, feelings of incapacity, uncertainties, and fears. When the person begins to present high levels of pain and this becomes chronic, little by little the individual tends to isolate themselves and live in search of medical treatments for the necessary care. From this new life scenario, there is a great tendency for the individual to minimize his or her social relations, to stay away from work, from the family environment, and from activities that generate pleasure, and this can interfere in the mental health and possibly become a predictor of the appearance of possible mental disorders, such as depression, stress, and anxiety[50].

The increased life expectancy of Brazilians must be considered, since the older they are, the more cases of diseases are prone to cause CP, as well as a possible relationship between hypocortisolism and the evolution of pain. All these findings can subsidize public health policies and policies focused on the care of people with CP.

CONCLUSION

Moderate/severe CP was associated with methylation of the CpG42 site of the NR3C1 gene, with increased age and low cortisol levels.

Thus, the findings suggest epigenetic involvement in the NR3C1 gene methylation in association with CP and suggest the need to search for new evidence regarding the mechanisms that explain CP, especially from the epigenetic point of view, because they may bring subsidies for CP prevention and control, targeting patients with the profile found in the present study, which can be considered predictive for the occurrence of CP.

AUTHORS' CONTRIBUTIONS

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Data Collection, Conceptualization, Project Management, Research, Methodology, Writing - Preparation of the original, Visualization
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