Application of vascular photobiomodulation therapy in individuals with low back pain and its relationship with global methylation

Aplicação da terapia por fotobiomodulação vascular em indivíduos com lombalgia e sua relação com a metilação global

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ABSTRACT

BACKGROUND AND OBJECTIVES: Low back pain is among the most disabling conditions worldwide, and among the epigenetic factors, methylation in CpG islands of gene promoter regions can modulate gene expression, potentially correlating with the development of the disease and providing insights into the choice of treatment. The objective of this study was to assess the efficacy of therapy using modified ILIB related to DNA methylation processes in low back pain. Secondary objectives of this study included investigating pain intensity, gender, sociodemographic data, and physical-functional profile.

METHODS: This prospective study was conducted in a municipality in the southern region of Brazil. The sample consisted of 30 participants of both genders, with an average age of 41.77 years. The following aspects were analyzed: anthropometric characteristics, global methylation using the ELISA method, pain level, physical activity level, functional disabilities, and hesitancy level related to work and physical activity-related activities.

RESULTS: A statistically significant association was observed between methylation levels before and after treatment application for the experimental and placebo groups ($p < 0.005$), demonstrating a mean responsiveness between methylation and treatment ($d = 0.5$). However, there were no other statistically significant associations correlated with the other work variables.

CONCLUSION: The results obtained in this study suggest the need for further research related to the identification of specific genes in methylation, as well as the standardization of dosimetry used for transcutaneous ILIB laser application in the radial artery.

Keywords: **Keywords**: DNA methylation, Epigenetics, Low back pain, Low intensity light therapy, Pain.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A lombalgia está entre as condições mais incapacitantes no mundo e; dentre os fatores epigenéticos, a metilação em ilhas CpG de regiões promotoras de genes pode modular a expressão gênica permitindo uma possível correlação ao desenvolvimento da doença, como também pode trazer esclarecimentos a respeito do tratamento a ser escolhido. O objetivo deste estudo foi verificar a eficácia da terapia através do uso do ILIB modificado relacionada ao processo de metilação de DNA na lombalgia. Os objetivos secundários deste estudo foram a investigação da intensidade da dor, sexo, dados sociodemográficos e perfil físico-funcional.

MÉTODOS: Este estudo, desenvolvido em um município da região sul do Brasil, caracteriza-se como prospectivo. A amostra deste estudo foi composta por 30 participantes, de ambos os sexos, com idade média de 41,77 anos. Foram analisados os seguintes aspectos: características antropométricas, metilação global através do método ELISA, nível de dor, nível de atividade física, incapacidades funcionais e nível de hesitação para realizar atividades relacionada ao trabalho e atividade física.

RESULTADOS: Observou-se associação estatisticamente significativa entre os níveis de metilação antes e a após aplicação do tratamento para grupo experimental e placebo ($p<0,005$) demostrando uma média responsividade entre as variáveis metilação e tratamento ($d=0,5$). No entanto, não houve nenhuma outra associação estatística correlacionada as demais variáreis do trabalho.

CONCLUSÃO: Os resultados obtidos neste estudo sugerem que há necessidade mais estudos relacionados a identificação de genes específicos na metilação, além da necessidade de padronização de dosimetria utilizadas para aplicação do laser ILIB de forma transcutânea, em artéria radial.

Descritores: Dor, Epigenética, Lombalgia, Metilação do DNA, Terapia com luz de baixa intensidade.

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HIGHLIGHTS

- Changes related to the methylation process and its relationship with modified ILIB treatment.
- Modified ILIB treatment and its relationship with pain.
- There is no epigenetic consensus for methylation alterations and low back pain, and more studies are needed to clarify their correlation with the disease.

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INTRODUCTION

Low back pain (LBP) is a complex condition and can be classified according to its mechanism as nociceptive, neuropathic and nociplastic. It is worth noting that the perception of pain is different from nociception, as it involves not only the activation of nerve fibers, but also emotional, cognitive, behavioral, genetic and epigenetic factors[1,2]. Thus, different epigenetic alterations may be related to LBP, including methylation of CpG islands in gene promoter regions, which can modulate gene expression without altering the DNA sequence[2]. Some genes, such as CELSR1, NAV1, MINK1 and KIF11, have already shown specific methylated regions, and these have been correlated with pain process, cell-cell participation/migration and neural differentiation[3].

In addition, the methylation of genes involved in the inflammatory response and tissue regeneration can affect susceptibility to LBP and the ability to recover from injuries. Understanding the epigenetic mechanisms underlying LBP can help to identify new therapeutic targets and personalize treatment for patients with different epigenetic profiles[4,5]. Despite the existence of various types of lasers for treating pain and inflammation, the modified application ILIB laser or modified ILIB (non-invasive laser irradiation of blood by transcutaneous means) is a therapeutic resource that emits photons of light that are absorbed by photoreceptors in the body, causing a change in electron transport and mitochondrial membrane potential, thus impacting the mitochondrial synthesis of ATP[6]. The mitochondria respond to treatment by activating transcription factors, modulating the expression and synthesis of pro-inflammatory proteins[7]. However, the epigenetic mechanisms involved in this process are still unknown, as is the efficacy of this treatment in patients with low back pain, and this is the first study to carry out such analyses.

Realizing a possible correlation between the inflammatory process and the treatment carried out by non-invasive laser irradiation of blood with LBP, there is a need for a more in-depth understanding of the aspects related to treatment effectiveness, as well as its relationship with epigenetic alterations verified through the methylation process. The secondary objectives of this study were to investigate pain intensity before and after treatment, gender and sociodemographic data to characterize sample heterogeneity and physical-functional profile.

METHODS

This work was a pilot study involving 30 adult individuals, aged between 18 and 59, who were recruited via telephone, e-mail or through publications on websites belonging to the universities that conceived this project. This age group was selected to provide data from a more heterogeneous sample in terms of general health, work status and contributing factors to LBP development and worsening.

This study included individuals who had LBP without an idiopathic cause and who were working. Those with the following characteristics were excluded: any type of neurological disease; cardiorespiratory, metabolic and orthopedic diseases; vestibulopathy; labyrinthine crises; mental problems; attention and speech disorders; any problem that could interfere with the physical and functional tests; had undergone some type of locomotor system surgery; used painkillers, muscle relaxants or anti-inflammatories during the study period; were pregnant; and did not volunteer spontaneously.

All the data and assessments carried out on the volunteers took place in laboratories specifically designed for such activities. This study was approved by the Ethics Committee for Research Involving Human Beings under Opinion Number 5.344.523, in accordance with Resolution 466/12 of the Brazilian National Health Council (*Conselho Nacional de Saúde*).

This study was carried out in stages. Initially, the participants were given information about the research (they were informed about the objectives of the study and the procedures adopted for data collection and, once they were aware, they signed the Free and Informed Consent Term, agreeing to take part in this research). After the participants were aware of and agreed to the terms of the study, the interview and data collection were carried out (the volunteers were submitted to the assessment instruments and, in order to characterize the sample, questions were asked such as: age, gender, smoking, alcoholism, and use of drugs; in addition, anthropometric measurements were taken for each participant: body weight and height. This data was collected for a better understanding of the sample. At this stage, the biological samples were also obtained pre-realization of the modified ILIB); the application of treatment: the participants were separated into two groups, an experimental group and a placebo group. The selection of participants belonging to the groups was defined through the random selection of pre-defined envelopes: 15 envelopes for treatment and 15 envelopes for placebo.

To apply the laser, the volunteers were seated on a chair with a backrest to accommodate the lumbar region and reduce fatigue; their feet were flat on the floor and their arms rested on a table in front of the patient. The treatment was applied with a separation between patient and laser, preventing visualization of the treatment. The region where the laser was applied was the left radial artery, for a period of 30 minutes.

All the individuals in the experimental group received 10 sessions of the modified ILIB on consecutive days, with a red laser, wavelength of 660nm and power of 100mW at a frequency of 1J/cm². All the application parameters were specific to the device and pre-established by the manufacturer (*DMC Equipamentos*). Placebo group had the same application position and the same number of sessions, but laser was switched off.

After the treatment, the participants were re-evaluated, answering the evaluation instruments again and having new biological material collected.

- The Fear Avoidance Beliefs Questionnaire (FABQ) is a questionnaire designed to measure levels of pain avoidance due to fear related to work and physical activity in patients with low back pain. The analysis instrument is subdivided into a four-item physical activity subscale (FABQ-Phy) and a seven-item work subscale (FABQ-Work). Each item is scored from 0 to 6 and added together to produce the score. The possible scores

range from 0 to 28 for FABQ-Phy and from 0 to 42 for FABQ-Work[11].

• Pain was measured using the Visual Analogue Scale (VAS), which was used to assess the intensity of LBP in patients. The scale has a score from zero (no pain) to 10 (worst imaginable pain)[8].

• The Roland Morris Questionnaire (RMQ) was used to assess functional disabilities. This questionnaire, validated for use in Brazil, aims to assess the functional disabilities of patients with LBP. It has 24 items that correlate functional capacity with the patient's painful symptoms. This list contains some phrases that people usually use to describe themselves when they have back pain. Each sentence ticked corresponds to one point, so the score ranges from zero to 24, and the higher the score obtained, the greater the influence of LBP on their functional capacities[9].

• The International Physical Activity Questionnaire (IPAQ) was used to check the average weekly time spent on physical activities of varying intensities (light, moderate and vigorous)[10]. It was applied in the long version, which covers the areas of work, transportation, domestic activities, leisure and time spent per week in sitting position[10].

• DNA was obtained from peripheral blood leukocytes, collected with EDTA, using the Pure Link Genomic DNA Extraction Kit (Invitrogen, Carlsbad, USA), according to the manufacturer's instructions. The extracted DNAs were stored in a freezer (80ºC) until analysis. The quality and quantity of DNA was assessed using absorbance analysis in a spectrophotometer (NanoDrop 2000 - Thermo Scientific) at 260nm and 280nm. DNA was then diluted in ultrapure water to obtain a final concentration of 50ng/uL.

Global methylation analysis was carried out in duplicate using the Imprint Methylated DNA Quantification kit (MDQ1, Imprint, Sigma-Aldrich) to detect relative levels of methylated DNA, based on the Enzyme Linked Immuno Sorbent Assay (ELISA) principle. A 96-well plate format was used. DNA samples were diluted in binding solution, and the amount of DNA used was calculated to obtain a final concentration of 50ng/µL. DNA binding was achieved by incubating 30 µL of diluted DNA at 37°C for one hour. A blocking solution was added and the samples were incubated again at 37°C for 30 minutes. The methylated DNA was then captured using capture antibodies detected by binding to the previously diluted detection antibodies. As stated by the kit manufacturer, after adding a development solution to the wells, a colorimetric reaction occurred[13]. Thus, color changes were monitored both after the addition of the development solution and 10 minutes after incubation, when the stop solution was added. Both a negative control (blank) and a control with 100% methylated DNA (positive control) were analyzed together with the DNA samples. The absorbance was measured at 450 nm. The percentage of global DNA methylation was calculated using the following formula: ([A450nmSample-A450nmBlank]/ A450nmMethylated control DNA-[A450nmBlank])X100[13].

Statistical analysis

To check the normality of the data obtained, the Shapiro-Wilk test was used, and after analysis, it was noted that the data had a value of p>0.05 and was considered normal, therefore parametric. The differences in the continuous variables between groups were assessed using Student's t-test and expressed as mean and standard deviation. Chi-square test or Fischer's exact test were used to analyze the association between dependent variables (treatment) and the other study variables: IPAQ, FABQ, VAS, RMQ, smoking and alcohol consumption. Cohen's d test was used to assess the size of the treatment effect; the effect size was interpreted as d=≤0.2 for small effect size; d>0.2≤0.7 for medium effect size; and d≥0.8 for large effect size. Categorical variables were expressed in absolute numbers and percentages. The statistical analyses were carried out in IBM SPSS Windows 24 (SPSS, Inc; Chicago, IL, USA) and the significance value adopted was p<0.05.

RESULTS

This study sample included a total of 30 participants, 12 men with an average age of 42±11.91 years and 18 women with an average age of 41.61±12.27 years. With regard to ethnicity, 50% of the volunteers were white, 40% brown and 10% black. Of the total number of participants, 80% did not use tobacco and 60% did not consume alcohol. With regard to physical exercise, 76.7% did not exercise at all, 16.6% exercised between one and three times a week and only 6.6% exercised between five and seven times a week (table 1).

Table 1. Demographic distribution and characteristics of the study population

Characteristics	n	%
Gender		
Male	12	40
Female	18	60
Age (years)		
21 - 30	7	23.33
31 - 40	8	26.66
41 - 50		13.33
50 - 58	11	36.66
Smoking		
No	24	80
Yes	6	20
Alcohol consumption		
No	18	60
Yes	12	40
Physical exercise		
Does not perform	23	76.7
1-3x/week	5	16.6
5-7x/week	2	6.66
Ethnicity		
White	15	50
Brown	12	40
Black	3	10

Table 2. Association between treatment and the other variables presented in this study

Characteristics		Treatment (%)	Placebo (%)	Pearson's Chi-squared test	p-value
IPAQ – pre	Sedentary	6.7	6.7	4.629	0.328
	Intermediary	43.3	46.6		
	Active	60	46.6		
IPAQ – post	Sedentary	26.7	13.3	3.818	0.431
	Intermediary	20	46.7		
	Active	53.4	40		
Smoking	Yes	73.3	86.7	0.833	0.361
	No	26.7	13.3		
Alcohol consumption	Yes	26.7	53.3	2.222	0.136
	No	73.3	46.7		
VAS - pre	0 to 3	30	23.1	12.623	0.179
	4 to 7	50	37.7		
	8 to 10	20	38.5		
VAS - post	0 to 3	80	69.3	3.487	0.837
	4 to 7	20	23.1		
	8 to 10	0	7.7		
FABQ -phys	0 - 12	80	84.7	9.92	0.357
	13 - 24	20	15.3		
FABQ -work	0 - 12	50	23.1	16.896	0.392
	13 - 24	20	53.9		
	25 - 42	30	23.1		
RMQ - pre	0 - 13	60	46.2	16.082	0.308
	14 - 24	40	53.9		
RMQ - post	0 - 13	90	53.9	18.253	0.250
	14 - 24	10	46.2		

IPAQ-pre = International Physical Activity Questionnaire before laser application; IPAQ-post = International Physical Activity Questionnaire after laser application; VAS-pre = Visual Analogue Scale before laser application; VAS-post = Visual Analogue Scale after laser application; FABQ-phys = Fear Avoidance Beliefs Questionnaire related to physical activity; FABQ-work = Fear Avoidance Beliefs Questionnaire related to work; RMQ-pre = Roland Morris Questionnaire before laser application; RMQ-post = Roland Morris Questionnaire after laser application.

Table 3. Relationship between the methylation variable and modified ILIB laser application

	n	Mean (%)	Standard deviation	p-value
Placebo group				
Pre-treatment	13	32.24	14.85	0.00001
Post-treatment	13	24.74	18.43	
Experimental Group				
Pre-treatment	10	41.88	16.32	0.00002
Post-treatment	10	34.13	19.81	

As can be seen in table 2, there was no statistically significant change (p>0.05) in IPAQ, FABQ, VAS, MRQ, smoking and alcohol consumption variables after treatment with modified ILIB.

With regard to methylation, there was statistical significance between groups before and after application of the modified ILIB laser, both in treatment and control groups, as shown in table 3. There was a mean methylation of 32.24±14.85 for control group before laser application and a mean methylation of 24.74±18.73 for the same group after treatment (p<0.01); for treatment group there was a mean of 41.88±16.32 before laser application and 34.13±19.81 after laser application (p<0.01). The effect size of the treatment with modified ILIB laser was d=0.5, considered a medium effect size in relation to global DNA methylation.

There was no statistically significant correlation (p>0.05) between methylation and the other study variables (IPAQ, VAS, FABQ, RMQ, smoking and alcohol consumption).

DISCUSSION

This study investigated the effect of modified ILIB therapy on the DNA methylation process in individuals with low back pain. Although no correlation was found between the treatment and the other variables in this study (such as: improvement in functional disabilities through the RMQ; improvement in pain intensity through the VAS; or the time spent on varied physical activities, verified through the IPAQ), the levels of methylation pre- and post-application of the treatment showed significant differences, indicating an increase in expression in both experimental group and placebo group. Based on the effect size of the

treatment, the decrease in the percentage of global methylation showed a medium effect, indicating that the methylation intervention showed medium responsiveness.

DNA methylation generally silences genes when it occurs in the promoter and enhancer gene regions[14]. As DNA methylation plays an important role in gene expression, it can also contribute to the occurrence of epigenetic changes, contributing to the development of various diseases[15], such as low back pain, in the case of NF-KB activation, which acts on inflammation by transcribing pro-inflammatory genes, thus aggravating intervertebral disc degeneration, which occurs when there is hypermethylation of the CARD14, EFHD2 and RTKN2 genes[16].

Other studies, also related to hypermethylation[17,18], evaluated the relationship between TRPA1 methylation and pain in different circumstances; the first study compared neuropathic pain in individuals with chronic pain and preoperative patients for thoracic surgery; while the second study analyzed patients with Crohn's disease, related to inflammation, and healthy individuals; in both studies, the researchers found an association between pain and methylation level.

According to one study[19], there are some factors that can influence the methylation analysis process, such as the sample group being the same or in the case of analysis of different biological samples (saliva and blood) bringing hyper- and hypomethylation results. It was also emphasized that some methylation investigation methods can identify areas of interest with greater sensitivity. Thus, methylation levels and the identification of the genes involved in the analysis process are important for improving understanding and clarifying the data obtained, since different genes can methylate differently[19].

One cancer-related study[20] found that L3MBTL1 methylation was associated with lower gene expression and lower risk of cancer recurrence, making it a protective factor.

In this way, it is possible for different genes to be hypermethylated and act in different ways. Study[21] highlighted the CD44 gene, this gene acts on interleukin-4 (IL-4) and Interferon-γ (IFN-γ). Activation of CD44 encephalitogenic T cells by the myelin oligodendrocyte glycoprotein (MOG) peptide led to demethylation of the promoter region of IFN-γ and interleukin-17 (IL-17), and hypermethylation of the IL-4 gene promoter and Forkhead winged helix transcription factor-3 (Foxp3). Similar activation of CD44-deficient encephalitogenic T cells led to hypermethylation of IFN-γ and IL-17a genes and demethylation of IL-4 and Foxp3 gene promoters. The researchers concluded that CD44 gene reciprocally regulates the differentiation of encephalitogenic T cells, through DNA methylation of the promoters of IFN-γ/IL-17a and IL-4/Foxp3 genes[21].

In the present study, global methylation was assessed using the ELISA method, and it was not possible to identify which genes were or were not methylated, making it impossible to investigate the expression of specific genes which, for example, could code for pro- or anti-inflammatory cytokines. However, this study highlighted the difference between the groups before and after treatment; and although there was a difference in these groups, even in the placebo group, there was global DNA hypomethylation. Therefore, an average responsiveness was shown. However, the identification of the genes could have contributed to the distinction and further clarification of the results, since hyper/hypomethylation can occur in different genes on different occasions, as has been seen in previous studies, and even in a placebo effect different genes could be expressed[19-21].

Taking into account the choice of treatment - vascular photobiomodulation (VPBM) or modified ILIB - it is known that the application of this laser acts on the absorption of photons by photoreceptors, such as the cytochrome C oxidase enzyme, leading to an increase in electron transport and mitochondrial membrane potential, and therefore an increase in mitochondrial ATP synthesis[6,7]. The mitochondria respond to treatment by activating transcription factors, modulating the expression and synthesis of pro-inflammatory proteins and reducing pain[8]. Despite this, this study found no improvement in pain intensity (assessed by VAS) after treatment. This result was also found in a study[22] in which photobiomodulation therapy was not superior to the placebo group in improving pain and disability in patients with chronic non-specific LBP. Another study[23] also reported similar results, indicating no difference between placebo and experimental groups in its analysis.

In other studies[24,25] the application of modified ILIB has shown positive responses, such as: improvement in chronic systemic diseases, modulation of inflammation and reduction in the levels of pro-inflammatory cytokines[24-26].

In addition, in a systematic review[25] on modified ILIB and ILIB, researchers highlighted the problem of dosimetry, where very low doses may not show responses in the irradiated tissue, while very high doses may cause inhibition, as well as issues related to irradiation time and power. However, there is no consensus on the doses to be used, thus hindering the use of low-power laser treatment[25].

This study found statistical significance between pain and treatment; the other variables showed no correlation. Fear, level of physical activity and functional incapacities remained unchanged, raising questions about the perception of pain and the treatment offered. Since people with chronic pain tend to adopt avoidance behaviors, they end up developing a fear of performing certain activities, generating a greater degree of disability. The period of treatment offered in this study might also not provide the necessary security to break the pattern established by the patients themselves, so the variables would not show statistically significant differences[26].

It is therefore necessary to be clearer about the dosimetry to be used, the length of treatment and the specificity of the methylated genes to be assessed, since failure to identify these does not make it clear whether pro- or anti-inflammatory mechanisms are at work in the different groups.

CONCLUSION

The results of this study showed global DNA hypomethylation between the pre- and post-application of modified ILIB for the placebo and experimental groups, demonstrating average respon-

siveness between the methylation and treatment variables. Further studies on the subject and the identification of specific genes are needed in order to obtain greater clarity from the data collected.

AUTHORS' CONTRIBUTIONS

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Statistical Analysis, Data Collection, Research, Methodology, Writing - Preparation of the Original, Writing - Review and Editing, Software
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REFERENCES

1. Knezevic NN, Candido KD, Vlaeyen JWS, Van Zundert J, Cohen SP. Low back pain. Lancet. 2021;398(10294):78-92.
2. Suntsov V, Jovanovic F, Knezevic E, Candido KD, Knezevic NN. Can implementation of genetics and pharmacogenomics improve treatment of chronic low back pain? Pharmac. 2020;12(9):894.
3. Aroke EN, Jackson P, Meng L, Huo Z, Overstreet DS, Penn TM, Quinn TL, Cruz-Almeida Y, Goodin BR. Differential DNA methylation in Black and White individuals with chronic low back pain enrich different genomic pathways. Neurobiol Pain. 2022;25(11):100086.
4. Adhikari B, Starkweather A, Xu W, Acabchuk Rl, Ramesh D, Eze B, Yang Y, Yang Gs, Walker J, Laubenbacher R, Park CL. A feasibility study on yoga's mechanism of action for chronic low back pain: psychological and neurophysiological changes, including global gene expression and DNA methylation, following a yoga intervention for chronic low back pain. Pilot Feasibility Stud. 2022;(8):142.
5. Tomé RFF, Silva DFB, Dos Santos CAO, Vasconcelos Neves G, Rolim AKA, Castro Gomes DQ. ILIB (intravascular laser irradiation of blood) as an adjuvant therapy in the treatment of patients with chronic systemic diseases-an integrative literature review. Lasers Med Sci. 2020;35(9):1899-907.
6. Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophys. 2017;4(3):337-61.
7. Zein R, Selting W, Hamblin MR. Review of light parameters and photobiomodulation efcacy: dive into complexity. J Biomed Opt. 2018;23:1.
8. Guyatt GH, Townsend M, Berman LB, Keller JL. A comparison of Likert and visual analogue scales for measuring change in function. J Chronic Dis. 1987;40(12):1129-33.
9. Roland M, Fairbank J. The Roland-Morris Disability Questionnaire and the Oswestry Disability Questionnaire. Spine (Phila Pa 1976). 2000;25(24):3115-24.
10. Benedetti TRB, Antunes PC, Rodrigues-Añez CR, Mazo GZ, Petroski EL. Reprodutibilidade e validade do questionário internacional de atividade física (IPAQ) em homens idosos. Rev Bras Med Esporte. 2007;13(1):11-6.
11. Cleland JA, Fritz JM, Brennan GP. Predictive validity of initial fear avoidance beliefs in patients with low back pain receiving physical therapy: is the FABQ a useful screening tool for identifying patients at risk for a poor recovery? Eur Spine J. 2008;17(1):70-9.
12. Gryzinska M, Blaszczak E, Strachecka A, Jezewska-Witkowska G. Analysis of age-related global DNA methylation in chicken. Biochem Genet. 2013;51(7-8):554-63.
13. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13(7):484-92.
14. Jin Z, Liu Y. DNA methylation in human diseases. Genes Dis. 2018;5(1):1-8.
15. Cao G, Yang S, Cao J, Tan Z, Wu L, Dong F, Ding W, Zhang F. The role of oxidative stress in intervertebral disc degeneration. Oxid Med Cell Longev. 202212;2022:2166817.
16. Takenaka S, Sukenaga N, Ohmuraya M, Matsuki Y, Maeda L, Takao Y, Hirose M. Association between neuropathic pain characteristics and DNA methylation of transient receptor potential ankyrin 1 in human peripheral blood. Medicine (Baltimore). 2020;99(8):e19325.
17. Gombert S, Rhein M, Winterpacht A, Münster T, Hillemacher T, Leffler A, Frieling H. Transient receptor potential ankyrin 1 promoter methylation and peripheral pain sensitivity in Crohn's disease. Clin Epigenetics. 2019;12(1):1.
18. Voisin S, Eynon N, Yan X, Bishop DJ. Exercise training and DNA methylation in humans. Acta Physiol (Oxf). 2015;213(1):39-59.
19. Satoh K, Ginsburg E, Vonderhaar BK. Msx-1 and Msx-2 in mammary gland development. J Mammary Gland Biol Neoplasia. 2004;9(2):195-205.
20. Li X, Xiao B, Chen XS. DNA methylation: a new player in multiple sclerosis. Mol Neurobiol. 2017;54(6):4049-59.
21. Guimarães LS, Costa LDCM, Araujo AC, Nascimento DP, Medeiros FC, Avanzi MA, Leal-Junior ECP, Costa LOP, Tomazoni SS. Photobiomodulation therapy is not better than placebo in patients with chronic nonspecific low back pain: a randomised placebo-controlled trial. Pain. 2021;162(6):1612-20.
22. Ay S, Doğan ŞK, Evcik D. Is low-level laser therapy effective in acute or chronic low back pain? Clin Rheumatol. 2010;29(8):905-10.
23. da Silva Leal MV, Lima MO, Nicolau RA, de Carvallho TMT, Abreu JAC, Pessoa DR, Arisawa EALS. Effect of modified laser transcutaneous irradiation on pain and quality of life in patients with diabetic neuropathy. Photobiomodul Photomed Laser Surg. 2020;38(3):138-44.
24. da Silva JGF, Dos Santos SS, de Almeida P, Marcos RL, Lino-Dos-Santos-Franco A. Effect of systemic photobiomodulation in the course of acute lung injury in rats. Lasers Med Sci. 2021;36(5):965-73.
25. Dos Santos Malavazzi TC, Fernandes KPS, Lopez TCC, Rodrigues MFSD, Horliana ACRT, Bussadori SK, Mesquita-Ferrari RA. Effects of the invasive and non-invasive systemic photobiomodulation using low-level laser in experimental models: a systematic review. Lasers Med Sci. 2023;38(1):137.
26. Xiao J. (Ed.) Physical exercise for human health. Adv Experimental Med Biol. 2020.