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THE LEVEL PRO-INFLAMMATORY AND ANTI-INFLAMMATORY BIOMARKERS IN PATIENTS WITH CHRONIC MECHANICAL LOW BACK PAIN UNDER PULSE RADIOFREQUENCY THERAPY

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Low back pain is a frequent and recurrent condition, often with a non-specific cause. Conventional treatment methods are generally insufficient in the treatment of chronic low back pain. The aim of the study was to estimate the level of IFN, IL-1, IL-6 (proinflammatory), IL-10, IL-4 (anti-inflammatory) and VEGF proteins in the serum of patients with chronic mechanical low back pain under Pulse radiofrequency (PRF) therapy. The study was carried out on 40 patients 20-60 years old, diagnosed with chronic low back pain for at least 4 months, primary complaint on lumbosacral low back pain, pain intensity VAS (visual analog scale) score of 5 and above, not responding well to conservative treatment (analgesic drugs, physiotherapy, etc.). Therapeutic Radiofrequency applications were carried out with an RF generator (RFG 3C Plus, Radionics). Blood samples were taken 1 day before interventional treatment (control), then 1 day (group1) and 15 days (group 2) after. The serum level of IFN, IL-1, IL-6, IL-10, IL-4 and VEGF l was analyzed with ELISA test. It was shown that as a result of PRF treatment the level of IL-1 was decreased while the levels of IL-4 and IL-6 were increased. It was concluded that the increase in serum levels of proinflammatory cytokines may be correlated with the severity of pain and that the increase in the level of anti-inflammatory cytokines reduces pain by reducing inflammation.

K e y w o r d s: chronic low back pain, radiofrequency therapy, cytokines.

Low back pain is a frequent and recurrent condition, often with a non-specific cause. Most non-specific acute low back pain resolves within a few weeks with or without treatment. Diagnostic work should focus on proving systemic or pathological causes. Treatment of chronic nonspecific low back pain includes a multidisciplinary approach aimed at preserving function and preventing disability [1, 2].

The lifetime prevalence of low back pain is 80%. In cases that do not heal within two months, a tendency to become chronic emerges. The prevalence of the disease leads to high treatment costs and job loss. Chronic low back pain is among the top causes of disability in the population under the age of 45. Conventional treatment methods are generally insufficient in the treatment of chronic low back pain.

Lumbosacral radicular pain, which is included in the classification of low back pain, is characterized by pain originating from one or more lumbar or sacral dermatomes, reflected to the low back and leg, and loss of function. Its incidence in the community is around 10-25%. It is the most common cause of neuropathic pain. In the acute condition, 60% of patients recover within 12 weeks. However, 30% of the pain becomes chronic and lasts longer than 3 months. If the pain cannot be controlled with pharmacological treatment, behavioral approaches and physical therapy, interventional pain treatment methods come to the fore.

Pulse radiofrequency (PRF) therapy is the intermittent application of high-frequency electric current at a temperature not exceeding 42 degrees. Thus, nerve damage is prevented and pain control is provided. Dorsal root ganglion blockade is a safe ap-

proach in terms of neurological complications when performed with PRF and is a highly successful algological intervention in the treatment of lumbosacral radicular pain. After this treatment, the analgesic requirements of the patients decrease and their functional quality of life increases. Dorsal root ganglion plays an important role in radicular pain [3].

In experimental studies, early and late increase in c-Fos release occurs in the dorsal horn with PRF treatment [4, 5]. An increase in ATF3 in the dorsal horn causes cell stress [6]. Treatment of a single dermatome causes similar effects in neighboring dermatomes. This is because the Lissauer tract transmits deep sensory impulses from the substantia gelatinosa to adjacent segments. In addition, A delta and C fibers also strengthen the same effect.

Lin et al. [7] monitored the chronic inflammation responses of chemokines via beta chemokine receptor 2 (CCR2) in the chronic compression model of DRG L4 and L5 and showed that there was a significant upregulation of CCR2 in DRGs. This was confirmed by another study by McKay et al. [8] showing a sustained inflammatory response of lumbosacral DRGs involving macrophages and T lymphocytes in thoracic spinal cord injury [8].

In chronic low back pain due to mechanical causes, examination findings and radiological images are generally used to define the disease. However, inflammatory and proinflammatory agents and hormones are thought to be important tools in revealing the cause and severity of the pathology. Cytokines are the leading factors affecting the spinal cord. Cytokines are molecules in protein or glycoprotein structure that provide intercellular communication in cell growth, tissue repair, cell remodeling and regulation of immune response. Cytokines are produced during the activation of innate and adaptive immunity and serve to determine the nature of the adaptive immune response by initiating the inflammatory response [9]. They are divided into two groups as pro-inflammatory and anti-inflammatory. Proinflammatory cytokines; TNF-α, TNF-β, IL-1, IL-8, IL-12, IL-15, IL-17, IL-18, INF-γ are released at the onset of inflammation to initiate and maintain the immune response. Anti-inflammatory cytokines, on the other hand, are IL-4, IL-10, IL-11, IL-13, and they are secreted at later stages of inflammation and control the inflammatory response [10].

TNF-α is a cytokine mostly produced by activated monocytes and macrophages, and the balance of benefit and harm is determined by its production levels and its relationship with other mediators [11, 12]. TNF-α is an important indicator of inflammation [13-15]. Interferons are from a family of proteins that can suppress the viral response [16]. It is found in various cells in lymphoid tissues, active lymphocytes [17]. Interferon-gamma (IFN-γ), produced by T cells originating from the thymus, is important in macrophage activation [18, 19]. IFN is one of the earliest proinflammatory cytokines involving inflammatory responses [20, 21]. Interleukin-4 (IL-4) is the main cytokine secreted by Th2, basophil, mast cells and [22]. IL-4 plays an important humoral regulatory role and adapts to the immune response, negatively regulating the production of proinflammatory cytokines [22]. Interleukin-4 is a Th2 protype that inhibits interferon gamma synthesis [23, 24]. Interleukin-10 (IL-10) is a pleiotropic cytokine that exhibits broad immune and anti-inflammatory activities [25]. During inflammation, IL-10 can be produced in large amounts by stimulation by myeloid cells and lymphocytes [26]. IL-17 determines target genes for autoimmune and chronic infections [27]. IL-17A is a proinflammatory cytokine that ensures the migration and differentiation of neutrophils [28, 29].

Vascular endothelial growth factor (VEGF) is one of these growth factors. They function in angiogenesis and lymphangiogenesis. Among the angiogenic molecules, VEGF is the most important and most emphasized, and it is also known as the vascular permeability factor (VPF) [30]. VEGF is an endothelial cell-specific mitogen that causes strong angiogenesis and vascular permeability [31]. VEGF also plays a role in the migration of endothelial cells, stimulating the release of matrix metalloproteases, which are responsible for the destruction of the extracellular matrix (ECM), and urokinase and tissue-type plasminogen activators. Thus, it facilitates invasion and metastasis [32]. VEGF was initially identified as a factor that increases vascular permeability. It regulates numerous biological functions of endothelial cells, cytokine synthesis and release, expression of molecules involved in thrombolytic and coagulation pathways, and smooth muscle cell hyperplasia [33]. In the light of this information, we aim to explain the relationship of IFN, IL-1, IL-6 (proinflammatory), IL-10, IL-4 (anti-inflammatory) and VEGF proteins on angiogenesis and cytokine release of interventional therapy in patients with chronic mechanical low back pain.

Materıals and Methods

Our study group consists of patients who applied to Manisa Celal Bayar University Faculty of Medicine, Department of Algology due to chronic low back pain. The study was carried out on 40 patients. Between the ages of 20-60, diagnosed with chronic low back pain for at least 4 months, primary complaint of lumbosacral low back pain, pain intensity VAS (visual analog scale) score of 5 and above, not responding well to conservative treatment (analgesic drugs, physiotherapy, etc.) patients were included in the study. In the clinical picture of the patients, radicular (reflected to the leg), contraction or stabbing dermatomal pain originating from the lumbar segments was detected. The type, level, and location of the lumbar spinal pathology causing the pain were confirmed by computed tomography or magnetic resonance imaging. Exclusion criteria: patient's reluctance to participate, mental disability or communication problems, infection, pregnancy, malignancy, psychiatric disorders, contraindications for neuraxial block, coagulation disorders, significant neurological defect, indication for back surgery, presence of local anesthetic and contrast substance allergy , motor loss, congenital anomaly-anatomical deformity. People who meet these standards and want to participate in the study were included in this study by signing a consent form. Pain treatment procedures were performed by the same physician (İ.T.).

Anti-inflammatory drug use of the patients was stopped 2 weeks before the interventional treatment for pain relief. Tramadol 2X37 mg (15 drops) was given orally for analgesia when necessary.

Our study group was planned as 3 groups, 1 day before the intervention, 1 day after the interventional treatment and 15 days after the interventional treatment.

Control blood samples were taken from all cases 1 day before the intervention. These blood samples were named as control group.

The RF administration procedure was performed using sterile techniques. With the patient in the prone position, 1% lidocaine was infiltrated into the skin. Correct placement of the needles was ensured by C-arm fluoroscopic imaging throughout the procedure. Radiofrequency applications were carried out with an RF generator (RFG 3C Plus, Radionics). Transforamen, from which the relevant peripheral nerve emerges, are marked in the accompaniment of fluoroscopy images. After needle insertion, 0.2 µml radiopaque contrast injection was observed, and it was observed that the needle tip was not vascularized and dispersed around the relevant peripheral nerve root. RF needles were selected with a 10 cm 22 gauge SMK-C10 electrode with a 5 mm active tip (Radionics, Burlington, MA). The impedance was monitored to be between 300 and 700 Ω to verify proper electrode placement. It was observed that patients' pain was re-stimulated at a rate of less than 0.5 V when sensory stimulation (50 Hz) was given with the RF electrode. A 6-minute, 45 V, 2 Hertz PRF wave was applied, with the electrode tip temperature not exceeding 42°C. Tramadol 2X37 mg (15 drops) was given orally for postoperative analgesia.

Blood samples were taken from all cases 1 day and 15 days after the interventional treatment. The blood samples taken after 1 day constitute the post-treatment group, that is, the 1st group. The blood samples taken 15 days after the treatment also constitute the second group. Our study was approved (Decision number: 15.03.2019/18) by the ethics committee of Manisa Celal Bayar University Faculty of Medicine

The collected blood was separated into serum and stored in the deep freeze at -80 degrees in the Department of Medical Biochemistry. Afterward, all of these collected blood samples were analyzed with the same analysis method for IFN, IL-1, IL-6, IL-10, IL-4 and VEGF levels by ELISA (R&D Systems, Minneapolis) test in the research laboratory of the Vocational School of Health Services.

Statistical analyzes were performed using Microsoft Excel and SPSS 15 computer programs. The distribution of the data will be analyzed with the Kolmogrov Smirnov test. Comparisons were made with the Mann Whitney U test for non-parametric distribution and Student's *t*-test for parametric distribution in both groups. If significant variability was detected, association was evaluated with Spearman or Pearson correlation tests.

Results

The study was carried out on 40 patients. However, since 2 patients did not apply to the hospital to complete the study, the data of 38 patients were evaluated. IFN, IL-1, IL-6, IL-10, IL-4 and VEGF serum levels were measured from the blood taken before the treatment, 1 day after the treatment and 15 days after the treatment from our patient group diagnosed with chronic low back pain, who underwent pulse radiofrequency application

to the dorsal root ganglia. The mean age of our patients was 53.2 ± 16.3 years, the mean height was 163.9 ± 11.8 cm, and the weight was 69.1 ± 13.0 kg. Serum samples from healthy donors/ reference ranges for healthy persons, stated by the ELISA kits manufacturer; VEGF: 28.1 – 1,800 pg/ml, IL-6: 12.5 – 800 pg/ml, IL-4: 0.3 – 16 pg/ml, IL-10: 7.8 – 500 pg/ml, IFN-γ: 15.6 – 1,000 pg/ml, IL-1: 0.1 – 8 pg/ml.

Human IFN-α serum level, which is one of the proinflammatory cytokines, was observed at similar rates before, 1 day and 15 days after the pain intervention, and no statistically significant change was detected. Human IL-6 is also a proinflammatory cytokine, and although the pre-treatment serum level did not show a statistically significant change 1 day after treatment, it increased significantly 15 days after treatment (Table 1).

Human IL-1 serum level, which is also among the proinflammatory cytokines, increased on the 1st day after the treatment. However, this height is not statistically significant. On the 15th day after the treatment, the serum Human IL-1 level was observed to be statistically significantly decreased compared to the pre-treatment values (Table 1).

Human IL-4 serum level, which is one of the anti-inflammatory cytokines, increased statistically significantly 1 day after the treatment and 15 days after the treatment compared to the values before the pain intervention. Human IL-10 serum level, which is one of the anti-inflammatory cytokines, was observed at similar rates before, 1 day and 15 days after the pain intervention, and no statistically significant change was detected (Table 2).

VEGF is also an anti-inflammatory cytokine, and although the pre-treatment serum level did not show a statistically significant change 1 day after treatment, it decreased significantly 15 days after treatment.

Discussion

Harmful stimuli that are strong enough to cause tissue lesions lead to the release of many algogenic and pro-inflammatory chemical mediators [34, 35]. Proinflammatory cytokines; TNF-α, TNF-β, IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, INF-γ are used to initiate and maintain the immune response are released at the onset of inflammation. Anti-inflammatory cytokines are VEGF, IL-4, IL-10, IL-11, IL-13 and they control the inflammatory response by being secreted at later stages of inflammation.

In this study, changes in serum levels of IFN, IL-1, IL-6 (pro-inflammatory), IL-10, IL-4 (anti-inflammatory), and VEGF were investigated in patients with chronic mechanical low back pain before and after treatment. These cytokines are the cytokines that give the first response during inflammation, and they also activate each other through the same pathway. Chronic mechanical low back pain is a term used to describe the symptoms of neuropathic pain in the distribution of a particular lumbar nerve root due to lumbar disc herniation, spinal stenosis,

Table 1. Serum levels of proinflammatory biomarkers before and after treatment

Proinflammatory biomarkers	Control	Group 1	Group 2	*P*
Human IFN-γ, ng/ml	12.3 ± 3.8	12.1 ± 4.5	13.6 ± 2.9	
Human IL-6, pg/ml	166.2 ± 53.4	156.9 ± 63.0	179.5 ± 51.1[&]	0.098
Human IL-1, pg/ml	675.3 ± 249.8	757.0 ± 191.3	590.7 ± 256.1[&]	0.005

Note: [&]Group 1

Table 2. Serum levels of anti-inflammatory biomarkers before and after treatment

Anti-inflammatory biomarkers	Control	Group 1	Group 2	*P*
Human IL-4, pg/ml	12.83 ± 4.3	16.12 ± 6.0*	15.06 ± 7.4*	0.020
Human IL-10, pg/ml	363.4 ± 109.5	377.0 ± 118.0	386.5 ± 95.1	
Human VEGF, ng/ml	26.7 ± 6.2	24.2 ± 9.0	21.88 ± 8.7*	0.030

Note: *Control group

facet hypertrophy or fibrosis. The pathophysiology of chronic mechanical low back pain includes mechanical, inflammatory and immunological factors that affect the function of the dorsal root ganglion (DRG).

Pulse radiofrequency (PRF) was developed as a modification of conventional radiofrequency ablation therapy. In the PRF, the quiescent phase is followed by short bursts of high voltage. Heat dissipation below 42°C to the target tissue is allowed. Pulse Radiofrequency therapy is a very safe pain treatment method with a low complication rate. A pulsed current is delivered by electrical radio waves to the dorsal root ganglion of the spinal functional unit causing pain. It has been suggested that it is more effective than conservative treatments for controlling radicular pain in chronic mechanical low back pain [36]. In a study by Cho et al., it was shown that the application of PRF in the dorsal root ganglion reduces microglial activity in the dorsal horn. The reduction in microglial activity can prevent the development of chronic pain by the release of cytokines and chemokines associated with neuropathic pain signals [37].

Few studies of patients with chronic mechanical low back pain have addressed the issue of detectable biomarkers in the acute phase. It has been shown that proinflammatory cytokines TNF-α, IL-6 and IL-8 and anti-inflammatory IL-4 and IL-10 play an important role in the inflammatory response following intervertebral disc herniation [38]. Increasing evidence supports the importance of immune activation in the etiology and progression of chronic low back pain [39]. Changes in proinflammatory cytokines such as IL-1, IL-2, IL-6 and TNF-α have been associated with changes in pain signaling pathways [40]. its place in the pathogenesis of mechanical low back pain remains unclear [38]. In addition, IL-1, IL-6 and TNF-α may be associated with the expression of matrix metalloproteinases, which can lead to herniation of intervertebral discs. Anti-inflammatory cytokines such as IL-4, IL-10 TNF, IL-1 and IL-6 are produced by activated macrophages and monocytes and can inhibit the synthesis of proinflammatory cytokines. IL-4 and IL-10 also suppress Th1 cells from releasing proinflammatory cytokines and inducing B lymphocyte differentiation [39, 41]. Schistad et al. showed that patients with high serum levels of the proinflammatory cytokine IL-6 had a weaker recovery in low back and leg pain [42]. Licciardone et al. compared the correlation of cytokine concentrations (IL-1/IL-6; IL6/IL-10; and IL-8/TNF-α) in asymptomatic and chronic mechanical low back pain patients. They found significant correlations between IL-1 and IL-6 concentrations and severe somatic dysfunction. They observed that IL-6 concentration correlated to a lesser degree with pain severity. Only TNF-a decreased after 12 weeks with manual therapy [43]. Koch et al. found a dose-response relationship between increasing cytokine concentrations (IL-1, IL-6, and TNF-α) and increased pain severity in 94 patients and 6 healthy controls with chronic neuropathic, nociceptive, or mixed pain for more than 6 months [44]. Vallejo et al. also observed that tumor necrosis factor-α and interleukin-6, which are proinflammatory cytokines, decreased after PRF treatment [45].

TNF-α, one of the proinflammatory cytokines, plays a role in increasing pain in the immune modulation of the spinal cord. Etanercept epidural, a TNF-α inhibitor, was administered to patients with sciatic pain. After 4 weeks, significantly more improvement was found compared to those who received dexamethasone [46]. In contrast, a blinded, placebo-controlled study found no significant clinical benefit 52 weeks after a single intravenous infusion of infliximab [47].

In the study of Chun Lin et al. [39], an increase in the anti-inflammatory cytokine IL-4 was shown between the treatments before and after auricular point acupressure in chronic low back pain. In addition, a decrease in the levels of proinflammatory cytokines IL-1α, IL-2 and IL-6 was also detected. It was observed that there was a moderate correlation between changes in pain intensity and changes in IL-1α and IL-2. Richards et al. [48] emphasized the importance of varying plasma concentrations of proinflammatory and anti-inflammatory cytokines in the pathophysiology of chronic mechanical low back pain. Among the 3 groups (healthy control, patients with chronic low back pain taking opioid analgesics for 3 months or more, patients with chronic low back pain not taking opioids for 3 months or more) various cytokines (IL-1, IL-2, IL-8) , IL-12p70, TNF-α, IFN-γ, IL-4, IL-10, IL-13, IL-6) found no difference in mean plasma concentrations [48].

Lee et al. [49] observed an increase in serum levels of proinflammatory biomarkers in patients with chronic low back pain, while a simultaneous decrease in anti-inflammatory cytokines was observed. In another study, it was shown that there is a positive correlation between IL-1 expression and

VEGF, NGF and BDNF protein expression in degenerative intervertebral disc patients [50]. During intervertebral disc degeneration, the release of cytokines, especially IL-1, causes neuronal proliferation and NGF (nerve growth factor) increases in the degenerated disc. IL-1 not only significantly increases neurotrophic factors, but is also known to express the angiogenic growth factor VEGF. NGF and VEGF released in response to IL-1 and potentially other cytokines target neurons and endothelial cells [51].

In our study, the serum level of IL-1, one of the proinflammatory cytokines, decreased as a result of DRG PRF treatment; It was determined that the serum level of IL-4, one of the anti-inflammatory cytokines, increased as a result of DRG PRF treatment. In addition, an increase in IL-6 plasma level was observed after interventional treatment. The increase in IL-6 will be beneficial in preventing inflammation by decreasing the IL-1 level. Decreased IL-1 also leads to a decrease in VEGF plasma level, preventing vascular remodeling.

As a result, we think that the increase in serum levels of proinflammatory cytokines may be correlated with the severity of pain and that the increase in the level of anti-inflammatory cytokines reduces pain by reducing inflammation. We believe that studies with larger samples conducted in specific patient groups and specific pain treatments will reveal which cytokines can be included in our clinical practice as biomarkers.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РІВЕНЬ ПРОЗАПАЛЬНИХ І ПРОТИЗАПАЛЬНИХ БІОМАРКЕРІВ У ПАЦІЄНТІВ ІЗ ХРОНІЧНИМ МЕХАНІЧНИМ БОЛЕМ У ПОПЕРЕКОВОМУ ВІДДІЛІ ХРЕБТА ЗА ПУЛЬСОВОЇ РАДІОЧАСТОТНОЇ ТЕРАПІЇ

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Біль у поперековому відділі хребта є частим і повторюваним станом, часто з неспецифічної причини. Традиційні методи лікування, як правило, недостатні для лікування хронічного болю у поперековому відділі хребта. Метою дослідження було оцінити рівень протеїнів IFN, IL-1, IL-6 (прозапальний), IL-10, IL-4 (протизапальний) та VEGF у сироватці крові пацієнтів із хронічним механічним болем у поперековому відділі хребта за допомогою імпульсної радіочастотної терапії (PRF). У дослідженні взяли участь 40 пацієнтів віком 20-60 років, у яких діагностовано хронічний біль у поперековому відділі хребта протягом щонайменше 4 місяців, інтенсивність болю за VAS (візуальна аналогова шкала) 5 балів і вище, і які слабко реагували на консервативне лікування (знеболюючі препарати, фізіотерапія та ін.). Радіочастотна терапія проводилася за допомогою радіочастотного генератора (RFG 3C Plus, Radionics). Зразки крові брали за 1 день до інтервенційного лікування (контроль), потім через 1 день (група 1) і через

15 днів (група 2) після початку лікування. Рівень IFN, IL-1, IL-6, IL-10, IL-4 та VEGF аналізували за допомогою ELISA-тесту. Показано, що в результаті PRF лікування рівень IL-1 знижувався, а рівні IL-4 та IL-6 підвищувалися. Було зроблено висновок, що підвищення рівня прозапальних цитокінів у сироватці крові може корелювати з інтенсивністю болю і, що підвищення рівня протизапальних цитокінів зменшує біль завдяки зменшенню запалення.

Ключові слова: цитокіни, хронічний біль у попереку, імпульсна радіочастотна терапія.

References

1. Golob AL, Wipf JE. Low back pain. *Med Clin North Am*. 2014; 98(3): 405-428.
2. Oberholzer A, Oberholzer C, Moldawer LL. Cytokine signaling – regulation of the immune response in normal and critically ill states. *Crit Care Med*. 2000; 28(4 Suppl): N3-N12.
3. Sayim NY. Deneysel kolit modelinde TNF-α blokerlerinin (infliximab ve etanercept) inflamasyon sürecine etkisi, Uzmanlık Tezi, Aydın. 2010.
4. Van Boxem K, van Bilsen J, de Meij N, Herrler A, Kessels F, Van Zundert J, van Kleef M. Pulsed radiofrequency treatment adjacent to the lumbar dorsal root ganglion for the management of lumbosacral radicular syndrome: a clinical audit. *Pain Med*. 2011; 12(9): 1322-1330.
5. Van Zundert J, de Louw AJA, Joosten EAJ, Kessels AGH, Honig W, Dederen PJWC, Veening JG, Vles JSH, van Kleef M. Pulsed and continuous radiofrequency current adjacent to the cervical dorsal root ganglion of the rat induces late cellular activity in the dorsal horn. *Anesthesiology*. 2005; 102(1): 125-131.
6. Higuchi Y, Nashold BS Jr, Sluijter M, Cosman E, Pearlstein RD. Exposure of the dorsal root ganglion in rats to pulsed radiofrequency currents activates dors*al horn lamina I and II neurons*. Neurosurgery. 2002; 50(4): 850-855.
7. Lin Q, Zou X, Willis WD. Adelta and C primary afferents convey dorsal root reflexes after intradermal injection of capsaicin in rats. *J Neurophysiol*. 2000; 84(5): 2695-2698.
8. McKay SM, McLachlan EM. Inflammation of rat dorsal root ganglia below a mid-thoracic spinal transection. *Neuroreport*. 2004; 15(11): 1783-1786.
9. Hamann W, Abou-Sherif S, Thompson S, Hall S. Pulsed radiofrequency applied to dorsal root ganglia causes a selective increase in ATF3 in small neurons. *Eur J Pain*. 2006; 10(2): 171-176.
10. Murch SH, Lamkin VA, Savage MO, Walker-Smith JA, MacDonald TT. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. *Gut*. 1991; 32(8): 913-917.
11. Karkucak M, Ursavaş A, Ocakoğlu G, Görükmez O, Yakut T, Ercan İ, Karadağ M. Analysis of TNF-alpha G308A and C857T Gene Polymorphisms in Turkish Patients with Obstructive Sleep Apnea Syndrome. *Türkiye Klinikleri J Med Sci*. 2012; 32(5): 1368-1373.
12. Demir S, Aykan FS, Öztuna D. Ankara Numune Eğitim Araştırma Hastanesi Göğüs Hastalıkları Kliniğinde son 8 yılda (2006-2013) TNF-alfa blokeri kullanan hastalara verilen latent tüberküloz tedavisi sonuçları. *Tuberk Toraks*. 2014; 62(4): 286-290.
13. Taoufik E, Tseveleki V, Euagelidou M, Emmanouil M, Voulgari-Kokota A, Haralambous S, Probert L. Positive and negative implications of tumor necrosis factor neutralization for the pathogenesis of multiple sclerosis. *Neurodegener Dis*. 2008; 5(1): 32-37.
14. Zhu TH, Nakamura M, Abrouk M, Farahnik B, Koo J, Bhutani T. Demyelinating disorders secondary to TNF-inhibitor therapy for the treatment of psoriasis: A review. *J Dermatolog Treat*. 2016; 27(5): 406-413.
15. Carvalho AF, Santos JR, Gentz R, Bonjardim CA, Golgher RR, Ferreira PCP, Kroon EG. Culture of human amniotic cells: A system to study interferon production. *Placenta*. 1998; 19(4): 307-314.
16. Hoshida MS, Gorjão R, Lima C, Daher S, Curi R, Bevilacqua E. Regulation of Gene Expression in Mouse Trophoblast Cells by Interferon-gamma. *Placenta*. 2007; 28(10): 1059-1072.
17. Zhang Y, Zhang B, Ye X, Yan Y, Huang L, Jiang Z, Tan S, Cai X. Electrochemical immunosensor for interferon-γ based on disposable ITO detector and HRP-antibody-conjugated nano gold as signal tag. *Mater Sci Eng C Mater Biol Appl*. 2016; 59: 577-584.
18. Sykam A, Gutlapalli VR, Tenali SP, Meena AK, Chandran P, Pratap DVS, Suneetha S, Suneetha LM. Association of tumor necrosis factor-alpha and interferon gamma gene polymorphisms and their plasma levels in

leprosy, HIV and other peripheral neuropathies. *Cytokine*. 2015; 76(2): 473-479.
19. Mohr DC, Genain C. Social support as a buffer in the relationship between treatment for depression and T-cell production of interferon gamma in patients with multiple sclerosis. *J Psychosom Res.* 2004; 57(2): 155-158.
20. Martin G, Moiola L, Brambilla E, Clementi E, Comi G, Grimaldi LME. Interferon-y induces T lymphocyte proliferation in multiple sclerosis via Ca^{2+}-dependent mechanism. *J Neuroimmunol*. 1995; 62(2): 169-176.
21. Nursal AF, Tekcan A, Kaya SU, Sezer O, Yigit S. Interleukin-1Ra rs2234663 and Interleukin-4 rs79071878 Polymorphisms in Familial Mediterranean Fever. *Gene.* 2016; 582(2): 173-177.
22. Urcelay E, Santiago JL, Mas A, Martínez A, de Las Heras V, Arroyo R, de la Concha EG. Role of interleukin 4 in Spanish multiple sclerosis patients. *J Neuroimmunol*. 2005; 168(1-2): 164-167.
23. Karakus N, Yigit S, Kurt GS, Cevik B, Demir O, Ates O. Association of interleukin (IL)-4 gene intron 3 VNTR polymorphism with multiple sclerosis in Turkish population. *Hum Immunol*. 2013; 74(9): 1157-1160.
24. Chen WCW, Lee BG, Park DW, Kim K, Chu H, Kim K, Huard J, Wang Y. Controlled dual delivery of fibroblast growth factor-2 and Interleukin-10 by heparin-based coacervate synergistically enhances ischemic heart repair. *Biomaterials.* 2015; 72: 138-151.
25. Ireland SJ, Monson NL, Davis LS. Seeking balance: Potentiation and inhibition of multiple sclerosis autoimmune responses by IL-6 and IL-10. *Cytokine*. 2015; 73(2): 236-244.
26. Qian X, Chen H, Wu X, Hu L, Huang Q, Jin Y. Interleukin-17 acts as double-edged sword in anti-tumor immunity and tumorigenesis. *Cytokine*. 2017; 89: 34-44.
27. Molvarec A, Czegle I, Szijártó J, Rigó J Jr. Increased circulating interleukin-17 levels in preeclampsia. *J Reprod Immunol.* 2015; 112: 53-57.
28. Guo S, Wu LX, Jones CX , Chen L, Hao CL, He L, Zhang JH. Allergic airway inflammation disrupts interleukin-17 mediated host defense against streptococcus pneumoniae infection. *Int Immunopharmacol.* 2016; 31: 32-38.
29. Toghianifar N, Ashtari F, Zarkesh-Esfahani SH, Mansourian M. Effect of high dose vitamin D intake on interleukin-17 levels in multiple sclerosis: a randomized, double-blind, placebo-controlled clinical trial. *J Neuroimmunol.* 2015; 285: 125-128.
30 Neuchrist C, Erovic BM, Handisurya A, Fischer MB, Steiner GE, Hollemann D, Gedlicka C, Saaristo A, Burian M. Vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 expression in squamous cell carcinomas of the head and neck. *Head Neck*. 2003; 25(6): 464-474.
31. Hovey RC, Goldhar AS, Baffi J, Vonderhaar BK. Transcriptional regulation of vascular endothelial growth factor expression in epithelial and stromal cells during mouse mammary gland development. *Mol Endocrinol.* 2001; 15(5): 819-831.
32. Hiratsuka S, Kataoka Y, Nakao K, Nakamura K, Morikawa S, Tanaka S, Katsuki M, Maru Y, Shibuya M. Vascular endothelial growth factor A (VEGF-A) is involved in guidance of VEGF receptor-positive cells to the anterior portion of early embryos. *Mol Cell Biol.* 2005; 25(1): 355-363.
33. Konukoğlu D, Turhan MS. Anjiyogenezin Temel Moleküler Mekanizmalari Ve Tümor Anjiyogenezi. *Cerrahpaşa Tip Dergisi.* 2005; 36(1): 42-48.
34. Baddack-Werncke U, Busch-Dienstfertig M, González-Rodríguez S, Maddila SC, Grobe J, Lipp M, Stein C, Müller G. Cytotoxic T cells modulate inflammation and endogenous opioid analgesia in chronic arthritis. *J Neuroinflammation.* 2017; 14(1): 30.
35. Stein C, Clark JD, Oh U, Vasko MR, Wilcox GL, Overland AC, Vanderah TW, Spencer RH. Peripheral mechanisms of pain and analgesia. *Brain Res Rev.* 2009; 60(1): 90-113.
36. Marliana A, Yudianta S, Subagya DW, Setyopranoto I, Setyaningsih I, Tursina Srie C, Setyawan R, Rhatomy S. The efficacy of pulsed radiofrequency intervention of the lumbar dorsal root ganglion in patients with chronic lumbar radicular pain. *Med J Malaysia*. 2020; 75(2): 124-129.
37. Cho HK, Cho YW, Kim EH, Sluijter ME, Hwang SJ, Ahn SH. Changes in pain behavior and glial activation in the spinal dorsal horn after

pulsed radiofrequency current administration to the dorsal root ganglion in a rat model of lumbar disc herniation: laboratory investigation. *J Neurosurg Spine*. 2013; 19(2): 256-263.
38. Dénes K, Arányi Z, Csillik A, Simó M, Debreczeni R, Tegze N, Bereczki D. Serum biomarkers in acute low back pain and sciatica. *Orv Hetil*. 2020; 161(13): 483-490.
39. Lin WC, Yeh CH, Chien LC, Morone NE, Glick RM, Albers KM. The Anti-Inflammatory Actions of Auricular Point Acupressure for Chronic Low Back Pain. *Evid Based Complement Alternat Med*. 2015; 2015: 103570.
40. Slade GD, Conrad MS, Diatchenko L, Rashid NU, Zhong S, Smith S, Rhodes J, Medvedev A, Makarov S, Maixner W, Nackley AG. Cytokine biomarkers and chronic pain: association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness. *Pain*. 2011; 152(12): 2802-2812.
41. Heijmans-Antonissen C, Wesseldijk F, Munnikes RJ, Huygen FJ, van der Meijden P, Hop WCJ, Hooijkaas H, Zijlstra FJ. Multiplex bead array assay for detection of 25 soluble cytokines in blister fluid of patients with complex regional pain syndrome type 1. *Mediators Inflamm*. 2006; 2006(1): 28398.
42. Schistad EI, Espeland A, Pedersen LM, Sandvik L, Gjerstad J, Røe C. Association between baseline IL-6 and 1-year recovery in lumbar radicular pain. *Eur J Pain*. 2014; 18(10): 1394-1401.
43. Licciardone JC, Kearns CM, Hodge LM, Bergamini MVW. Associations of cytokine concentrations with key osteopathic lesions and clinical outcomes in patients with nonspecific chronic low back pain: results from the OSTEOPATHIC Trial. *J Am Osteopath Assoc*. 2012; 112(9): 596-605.
44. Koch A, Zacharowski K, Boehm O, Stevens M, Lipfert P, von Giesen H-J, Wolf A, Freynhagen R. Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. *Inflamm Res*. 2007; 56(1): 32-37.
45. Vallejo R, Tilley DM, Williams J, Labak S, Aliaga L, Benyamin RM. Pulsed radiofrequency modulates pain regulatory gene expression along the nociceptive pathway. *Pain Physician*. 2013; 16(5): E601-E613.
46. Ohtori S, Miyagi M, Eguchi Y, Inoue G, Orita S, Ochiai N, Kishida S, Kuniyoshi K, Nakamura J, Aoki Y, Ishikawa T, Arai G, Kamoda H, Suzuki M, Takaso M, Furuya T, Toyone T, Takahashi K. Epidural administration of spinal nerves with the tumor necrosis factor-alpha inhibitor, etanercept, compared with dexamethasone for treatment of sciatica in patients with lumbar spinal stenosis: a prospective randomized study. *Spine*. 2012; 37(6): 439-444.
47. Korhonen T, Karppinen J, Paimela L, Malmivaara A, Lindgren KA, Bowman C, Hammond A, Kirkham B, Järvinen S, Niinimäki J, Veeger N, Haapea M, Torkki M, Tervonen O, Seitsalo S, Hurri H. The treatment of disc-herniation-induced sciatica with infliximab: one-year follow-up results of FIRST II, a randomized controlled trial. *Spine*. 2006; 31(24): 2759-2766.
48. Richards GC, Lluka LJ, MT Smith 3 , Haslam C, Moore B, O'Callaghan J, Strong J. Effects of long-term opioid analgesics on cognitive performance and plasma cytokine concentrations in patients with chronic low back pain: a cross-sectional pilot study. *Pain Rep*. 2018; 3(4): e669.
49. Lee JM, Song JY, Baek MJ, Jung HY, Kang H, Han IB, Kwon YD, Shin DE. Interleukin-1β induces angiogenesis and innervation in human intervertebral disc degeneration. *J Orthop Res*. 2011; 29(2): 265-269.
50. Watkins LR, Maier SF. Immune regulation of central nervous system functions: from sickness responses to pathological pain. *J Intern Med*. 2005; 257(2): 139-155.
51. Binch ALA, Cole AA, Breakwell LM, Michael ALR, Chiverton N, Cross AK, Le Maitre CL. Expression and regulation of neurotrophic and angiogenic factors during human intervertebral disc degeneration. *Arthritis Res Ther*. 2014; 16(5): 416.