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

I. TEKIN1, F. KOSOVA2✉

1Manisa Celal Bayar University Medical Faculty, Department of Anesthesia and Reanimation, Algology, Manisa, Turkey;
2Manisa Celal Bayar University School of Vocational Health Service, Department of Medical Biochemistry, Manisa, Turkey;
✉e-mail: fundakosova@gmail.com

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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 (group 1) and 15 days (group 2) after. The serum level of IFN, IL-1, IL-6, IL-10, IL-4 and VEGF 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.

Keywords: 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-
approach in terms of neurological complications when performed with PRF and is a highly successful al-
logogical intervention in the treatment of lumbosa-
cral radicular pain. After this treatment, the analge-
sic requirements of the patients decrease and their
functional quality of life increases. Dorsal root gan-
glion 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
Infomation [4, 5]. An increase in ATF3 in the dorsal
horn causes cell stress [6]. Treatment of a single der-
matome causes similar effects in neighboring der-
matomes. This is because the Lissauer tract trans-
mits deep sensory impulses from the substantia
gelatinosa to adjacent segments. In addition, A delta
fibers also strengthen the same effect.

In chronic low back pain due to mechani-
causal 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 glyco-
protein structure that provide intercellular commu-
nication in cell growth, tissue repair, cell remodeling
and regulation of immune response. Cytokines are
produced during the activation of innate and adap-
tive immunity and serve to determine the nature of
the adaptive immune response by initiating the in-
flammatory 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 acti-
vated monocytes and macrophages, and the balance of
benefit and harm is determined by its produc-
tion levels and its relationship with other mediators
[11, 12]. TNF-α is an important indicator of inflam-
matin [13-15]. Interferons are from a family of pro-
obins that can suppress the viral response [16]. It is
found in various cells in lymphoid tissues, active
lymphocytes [17]. Interferon-gamma (IFN-γ), pro-
duced by T cells originating from the thymus, is im-
portant 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 proinflamma-
tory 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 ac-
tivities [25]. During inflammation, IL-10 can be
produced in large amounts by stimulation by my-
eloid cells and lymphocytes [26]. IL-17 determines
target genes for autoimmune and chronic infections
[27]. IL-17A is a proinflammatory cytokine that en-
sures the migration and differentiation of neutrophils
[28, 29].

Vascular endothelial growth factor (VEGF) is
one of these growth factors. They function in angio-
genesis and lymphangiogenesis. Among the angio-
genic molecules, VEGF is the most important and
most emphasized, and it is also known as the vas-
cular 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 ex-
tracellular 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 perme-
ability. 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.
Materials 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 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 pro-inflammatory 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://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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15 днів (група 2) після початку лікування. Рівень IFN, IL-1, IL-6, IL-10, IL-4 та VEGF визначали 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 протизапальних цитокінів зменшує біль завдяки зменшенню запалення.

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

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