International Journal of Phytopharmacology
Research Article

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

PREVALENCE AND SEVERITY OF SYSTEMIC CYTOKINE RESPONSE IN LUMBAR DISC HERNIATION (CHRONIC LOW BACK PAIN) PATIENTS

S. Yoganathan[1], S. Ravichandran[2*]

[1]Associate Professor, Department of Orthopaedics, Swamy Vivekanandha Medical College Hospital and Research Institute, Elayampalayam, Tiruchengode, Namakkal Dt, Tamilnadu- 637205
[2]Professor and Head Department of Biochemistry, Swamy Vivekananda Medical College Hospital and Research Institute, Elayampalayam, Tiruchengode, Namakkal, Tamilnadu - 637205.

ABSTRACT
Lumbar Disc Herniation (LDH) is a mechanical and biochemical process, wherein disc comes in contact with spinal nerves causes compression, chemical irritation, inflammation and pain. Herniated disc tissue exhibits an inflammatory cellular infiltration and elevated pro-inflammatory cytokines. We compared cytokine levels in patients with chronic low back pain and in healthy subjects. Cytokine levels were measured using the enzyme-linked immunosorbent assay (ELISA) technique. Thirty five patients with low back pain (G1) and 17 healthy controls (G2) were selected. The pain duration among the herniated disc patients was 81 ± 99 months (median 34.5) and the pain intensity as measured using the numerical rating scale was 9.0 ± 1.7 (median 10). The location of the herniated intervertebral disc was at the L4-L5 levels in 60% of the patients and at the L5-S1 levels in 40%. Pain was continuous in 87% of the subjects, with a daily frequency in 87%. Chronic low back pain and disc herniation exhibited significantly higher levels of TNF-alpha and IL-6 compared with healthy controls. The conclusion is that serum proteins are considerable molecular markers of patients that develop constant pain after disc herniation.

Key words: Disc herniation, Nucleus pulposus, Interleukin-6, Annulus Fibrosus.

Corresponding Author: **S. Ravichandran** Email: dr.s.ravichand@gmail.com.

INTRODUCTION

Back ache is the main reason of disability exerting enormous burden on patients and the financial system. One of the most established disc pathologies that results in lumbar radicular ache is disc herniation (DH), defined so because of the displacement of intervertebral disc (IVD) fabric past the normal margins of the disc.

Access this article online

DOI: http://onlineijp.com/

Quick Response code

Received:05.02.2022 **Revised**:12.03.2022 **Accepted**:15.03.2022

LDH involves collectively a mechanical and biochemical process, wherein disc contacts with the spinal nerves causing compression, chemical irritation, inflammation and pain. Herniated disc tissue exhibits an inflammatory cellular infiltration and elevated pro-inflammatory cytokines,[2] that decrease during rest and raise in pain during activity.[3] LDH can be categorized as protrusions, extrusions, or sequestrations. Protrusions are wide-based herniations in which the diameter at the base of the herniation is wider than the diameter of the herniation in the canal. Extrusions have a narrow base, with a large herniation in the canal, and sequestrations are herniations in which there is no continuity between the herniation and the remaining intervertebral disc.

Normally, LDH has been associated with disruption of the annulus fibrosus (AF), extrusion of the nucleus pulposus (NP), and stimulation of nerve fibers, leading to

pain. On the other hand, more recently, authors suggested that disc herniation more frequently occurs in end plate junction failure than AF rupture. Herniated discs are found in 30–40% of asymptomatic people in imaging diagnostic tools.[4]

When nucleus pulposus leakage into the spinal canal after disc herniation may start immunological and inflammatory responses close to the nerve-roots that increase the activity in nociceptive pathways.[5]This inflammatory influence has been attributed to an elevation of interleukins (ILs), tumor necrosis factor (TNF), matrix metalloproteinases (MMPs), nitric oxide (NO), and prostaglandins (PGs) in or around the herniated disc.[6]

The aim of the present study is to investigate cytokines that could be present at abnormal levels in the blood and cerebrospinal fluid and include interleukin-8 (IL-8), interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and soluble TNF receptor (sTNF-R), the inflammatory factors in the serum of patients with persistent lumbar radicular pain after disc herniation.

Material and Method:

This analytical cross-sectional study conducted with 35 consecutive patients with last three months of back pain because of herniated disc disease. They were selected from the orthopedic department at Swamy Vivekananda Medical College Hospital and Research Institute, Elayampalayam, Tiruchengode and got approval from the institutional ethics committee. Informed consent statement collected from all patients. They were compared with 17 healthy controls (with age ranging from 20 to 60 years) from the hospital community, without any previous history of back pain, who were used as controls.

The analysis was confirmed by means of magnetic resonance imaging (MRI) or computed tomography (CT) imaging of the spine for all the patients. Their pain severity had to be ≥ 5 points on a numerical rating scale (NRS), which ranged from zero (no pain) to 10 (worst imaginable pain) in this study. Psychiatric disorders, systemic or inflammatory diseases, histories of allergy, presence of motor deficits, histories of blood dyscrasia, pregnancy, active infection, tumors, use of analgesic drugs during the preceding week, or inability to come to the hospital for evaluation were exclusion criteria in this study. All patients underwent standard history taking and physical examination and neurological findings of sensory and motor deficits and reflex dysfunction and the straight leg-raising test were also evaluated by means of clinical examination. All the data were registered to facilitate statistical analysis.

In this study, the sample size calculation was based on different studies in the literature (between 10 and 35 patients) and on the fact that normal individuals do not present circulating proinflammatory serum cytokines. Plasma was isolated by Ficoll density gradient centrifugation for 20 min at 800 g in Greiner Leucosep tubes.

Multiplex ELISA assay

The concentration of eight cytokines was analyzed in human plasma from patients with chronic back pain (n=35) and age matched pain-free healthy controls (n=30), by Bio-Plex Pro Cytokine Chemokine and Growth Factor Assay (Bio-Rad Laboratories AG, Cressier, Switzerland). Data were collected and analyzed using a Bio-Rad Bio-Plex 200 instrument equipped with Bio-Plex Manager software (Bio-Rad). We measured the concentrations of (IL)-2, IL-4, IL-6, IL-10, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), MCP1, and TNF-α.

Quantitative ELISA assays

Quantitative determination of CXCL6, IL-1β, CCL5, and stromal cell-derived factor 1 alpha (CXCL12) (Quantikine ELISA kits – R&D Systems, Abingdon, UK) in human plasma of patients with chronic back pain (n=35) and age matched pain-free healthy controls (n=17) was done with a DTX 880 Multiplex reader (Beckman Coulter, Nyon, Switzerland). Experiments were performed according to the respective manufacturer's protocols.

The variables did not present a normal distribution, and therefore nonparametric tests were used. The cytokine levels were compared between the study and control groups using the Mann-Whitney test. The Spearman coefficient was used to determine the relationship between cytokines and continuous variables. The chi-square or Fisher exact test was used when necessary, to test differences between proportions. The Statistical Package for the Social Sciences (SPSS) statistical software (version 10.0, SPSS Inc., Chicago, Illinois, US) was used for data analysis, and statistical significance was determined as P values < 0.05.

Results:

Thirty-five patients were enrolled in the study: The pain duration among the herniated disc patients was 81 ± 99 months (median 34.5) and the pain intensity as measured using the numerical rating scale was 9.0 ± 1.7 (median 10). The location of the herniated intervertebral disc was at the L4-L5 levels in 65% of the patients and at the L5-S1 levels in 40%. Pain was continuous in 87% of the subjects, with a daily frequency in 87%.

Table: 1 Patients' characteristics

	Gender	Age	Weight	Height
Group I(35)	Male -22 Female-13	45.8±9.0	69.7±9.0	165.1 ± 9.1
Group II(Controls 17)	Male -11 Female-6	37.5±4.9	62.3 ± 6.8	163.3 ± 6.7
	0.8330	0.1793	0.4860	0.93

62% were men. The mean age was 45.8 ± 9.0 years (median 43.0); the mean weight was 69.7 ± 9.0 kg (median 65.8); and the mean height was 165.1 ± 9.1 cm (median 167.0)

Table: 2 Neurological findings in the group of patients with herniated disc (G1; n = 35)

Reduced muscle strength	**5(14.2%)**
Hypoesthesia	**15(43%)**
Positive straight-leg raise test	**9(26%)**
Hyporeflexia	**6(17%)**

The neurological findings were: a positive straight-leg-raise test (26%); hyporeflexia (17%); hypoesthesia (43%); and reduced muscle strength (14.2%)

Table: 3 Serum cytokine levels in herniated disc patients (G1) and controls (G2)

Pg/ml	G1(35)	G2(17)	P
IL-beta	0.6±0.3	0.6-0.1	1
IL-6	5.1±3.0	0.9-0.5	0.01
TNF-alpha	6.4±2.3	1.8-0.7	0.01
sTNF-R	582±36	591±50	0.89

Serum levels of TNF-alpha and IL-6 were statistically higher in G1 ($P < 0.05$). There were no differences in IL-1 beta or sTNF-R levels between the groups ($P > 0.05$), according to the Mann-Whitney test.

The correlation coefficients between serum levels of TNF-alpha or IL-6 and pain intensity were, respectively, $rs = 0.29$, $P = 0.19$; $rs = 0.35$, $P = 0.15$; and in relation to duration of pain complaints were, respectively, $rs = 0.07$, $P = 0.87$; $rs = 0.10$, $P = 0.64$. There was also no correlation between the levels of proinflammatory cytokines and clinical parameters like age, weight and height ($P > 0.05$).

Discussion:

The purpose of this study was to assess the relationship between systemic ranges of inflammatory mediators and IVD herniation, to pick out the effects of herniation severity, pain intensity and period of signs and symptoms. The present study demonstrates that people with herniated lumbar intervertebral disc disease have increased serum levels of TNF-alpha and IL-6, compared with healthy controls.

The increase in serum IL-6 levels in individuals with an ongoing history of sciatic pain following discectomy have already been reported by Geiss A et al .[7] There is no such elevation has been found in subjects with disc herniation and sciatica in Brisby H, et al study.[8] Proinflammatory cytokines show circadian rhythms and variations in peripheral blood, and the differences can potentially be related to the following factors:

1) The time of the day at which the blood samples had been drawn, based on a have a look at that proven that IL-6 concentrations peaked within the night.
2) IL-6 is a cytokine that increases in concentration in response to stressful conditions and may be affected by any emotional changes or symptom amplification.[9]
3) Cytokines may be released in a time-ordered sequence.[10]
4) When an interleukin binds to its functional receptor, the complex is internalised.[11]
5) Cytokines are also potent stimulators of the hypothalamic-pituitary-adrenal (HPA) axis, a dysfunctional HPA axis response happening in some patients may result in elevated serum cytokine levels.

A recent study has demonstrated that inter vertebral disc cells may produce TNF-alpha and IL-1 beta immediately after the onset of disc herniation and these findings correlated with Nygaard et al. This study indicated that dissimilar types of disc herniation have different inflammatory properties.[12]

Koch et al.[13] showed that rising serum levels of proinflammatory cytokines such as IL-1 beta, IL-2, IL-6, interferon-gamma [IFN-gamma] and TNF-alpha associated with increasing pain intensity in patients with chronic pain which is correlated with present study.

High levels of proinflammatory cytokines had been pronounced in inflammatory and infectious sicknesses and can be correlated with disorder severity,[14] in this study. We did not find any other infection that

would provide an explanation for the excessive degrees of proinflammatory cytokines. On the other hand, our study group is small and, therefore, confounding factors couldn't be considered. Moreover, little is known concerning immune factors' effect on herniated discs pain. If the proinflammatory circulating cytokines are mediators of pain and neuropathological modifications in those sensory neurons, their inhibition constitutes an alternative to surgical remedy. This could lower the costs and postoperative complications.

The opportunities for pharmacological interventions targeting the neuro inflammatory and neuroimmune additives of various pathological conditions can be an interesting field of research. Thus, in addition, studies are wanted to clarify which of these methods are amenable to remedy and to decide the sensitivity and specificity of those observations for facilitating diagnoses, sickness monitoring, and prognoses.[15]

Conclusion:

Chronic low back pain and disc herniation exhibited significantly higher levels of TNF-alpha and IL-6 compared with healthy controls. We conclude that serum proteins possibly are considerable molecular markers of patients that develop constant pain after disc herniation.

REFERENCES

Gore M, Sadosky A, Stacey BR, Tai KS, Leslie D. The burden of chronic low back pain: clinical comorbidities, treatment patterns, and health care costs in usual care settings. Spine (Phila Pa 1976) 2012 5 15;37(11): E668–77.

Ma XL, Tian P, Wang T, Ma JX. A study of the relationship between type of lumbar disc herniation, straight leg raising test and peripheral T lymphocytes. Orthop Surg. 2010 2;2(1):52–7.

Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009 10 16;139(2):267–84.

Brinjikji W, Luetmer PH, Comstock B, Bresnahan BW, Chen LE, Deyo RA, et al. Systematic literature review of imaging features of spinal degeneration in asymptomatic populations. AJNR Am J Neuroradiol. 2015;36(4):811–6 PubMed PMID: 25430861. Pubmed Central PMCID: 4464797.

N. G. Egeland, A.Moen, L.M. Pedersen, H. Brisby, and J. Gjerstad, "Spinal nociceptive hyperexcitability induced by experimental disc herniation is associated with enhanced local expression of Csf1 and FasL," Pain, vol. 154, no. 9, pp. 1743–1748,2013.

P. Andrade, G. Hoogland, M. A. Garcia, H. W. Steinbusch, M.A. Daemen, and V. Visser-Vandewalle, "Elevated IL-1b and IL-6 levels in lumbar herniated discs in patients with sciatic pain,"European Spine Journal, vol. 22, no. 4, pp. 714–720, 2013.

Geiss A, Varadi E, Steinbach K, Bauer HW, Anton F. Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. Neurosci Lett. 1997;237(2-3):65-8.

Brisby H, Olmarker K, Larsson K, Nutu M, Rydevik B. Proinflammatory cytokines in cere¬brospinal fluid and serum in patients with disc herniation and sciatica. Eur Spine J. 2002;11(1):62-6.

Brydon L, Edwards S, Mohamed-Ali V, Steptoe A. Socioeconomic status and stress-induced increases in interleukin-6. Brain Behav Immun. 2004;18(3):281-90.

Cunha FQ, Poole S, Lorenzetti BB, Ferreira SH. The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. Br J Pharmacol. 1992;107(3):660-4.

Solari R, Smithers N, Kennard N, Ray K, Grenfell S. Receptor mediated endocytosis and intracellular fate of interleukin 1. Biochem Pharmacol. 1994;47(1):93-101.

Nygaard OP, Mellgren SI, Osterud B. The inflammatory properties of contained and noncon¬tained lumbar disc herniation. Spine (Phila Pa 1976). 1997;22(21):2484-8.

Koch A, Zacharowski K, Boehm O, et al. Nitric oxide and pro-inflammatory cytokines correla¬te with pain intensity in chronic pain patients. Inflamm Res. 2007;56(1):32-7.

Kalinkovitch A, Engelmann H, Harpaz N, et al. Elevated serum levels of soluble tumour necrosis factor receptors (sTNF-R) in patients with HIV infection. Clin Exp Immunol. 1992;89(3):351-5.

Huygen FJ, Niehof S, Zijlstra FJ, van Hagen PM, van Daele PL. Successful treatment of CRPS 1 with anti-TNF. J Pain Symptom Manage. 2004;27(2):101-3..