ORIGINAL ARTICLE

PRE-DIAGNOSTIC SERUM LEVELS OF INFLAMMATORY CYTOKINES IN PATIENTS WITH FIBROMYALGIA

Nusrat Tariq, Muhammad Rafiq*, Rahma Zahid Butt, Fatima Zahid Butt, Sulayman Waquar*, Arif Malik*

M. Islam Medical and Dental College Gujranwala, *Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan

Background: Fibromyalgia is characterised by fatigue, sleep disruption and pain perception. The aetiology of this syndrome is still unclear. Central sensitization is considered to be a major mechanism of action and other factors may be involved including hormonal, genetic and immunological factors. The objective of the present study was to evaluate the role of different interleukins and matrix metalloproteinases in the development of fibromyalgia. **Method:** Complete blood count (CBC) profile of 50 patients and 50 controls were measured on automated haematology blood analyser. The levels of Interleukins IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were measured using commercially available kits. Matrix metalloproteinases (MMP)-2, 7, and 9 were analysed by ELISA kit assay. **Results:** The mean values of IL-1, IL-2, IL-6, IL-7 and IL-9 were significantly increased in the patients with fibromyalgia when compared to control individuals. Elevated trends of IL-8, IL-17, IL-18, IL-1β and IL-1-RA were recorded in the patients with fibromyalgia as compared to control individuals respectively. The mean values of MMP-2, MMP-7 and MMP-9 were significantly increased in the patients with fibromyalgia as compared to control individuals. **Conclusion** The levels of inflammatory markers such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were significantly higher among the fibromyalgia patients as compared to controls. These inflammatory markers may have prominent role in the development and progression of disease condition.

Keywords: Fibromyalgia, matrix metalloproteinases, MMPs, interleukins

Pak J Physiol 2022;18(2):31–4

INTRODUCTION

Fibromyalgia belongs to the family of related disorders, which are called affective spectrum disorders. These disorders have genetic risk factors and physiological abnormalities that might be considered as central aetiology of the disease.[1] Affective spectrum disorders include a variety of psychiatric disorders such as bulima nervosa, premenstrual dysphoric disorder, major depressive disorder, panic disorder and various medical disorders (for example, cataplexy, migraine, irritable bowel syndrome). Pathophysiology of fibromyalgia includes multiple factors such as genetic disorders, autonomic nervous system disorders, environmental stressors and psychosocial variables. These factors are also linked with other disorders that commonly occur in fibromyalgia and are distinguished by emotional distress and recurrent or persistent pain.[1] Fibromyalgia may also occur with chronic inflammatory diseases including systemic lupus erythematosus, osteoarthritis and rheumatoid arthritis. The occurrence of one or more of these conditions may serve as the hallmark for the diagnosis and management of fibromyalgia. The patients suffering from fibromyalgia have increased sensitivity due to various stimuli including cold or heat as well as ischemic and mechanical pressure. In the fibromyalgia patients stimuli to the intensity of the pain varies among one to another.[2]

Fibromyalgia is distinguished as augmentation of sensory input, which is induced by central nervous system events that is linked with neuropathic pain complications such as central sensitization. Moreover, fibromyalgia patients also show abnormal levels of norepinephrine and serotonin, which are important neurotransmitters in pain inhibitory mechanism. In human and animal models of neuropathic pain, the source of nerve injury is identifiable, and pain perception might be decreased, once the source is eradicated. The study has reported the painful symptoms of fibromyalgia include abnormality in the descending pain inhibitory mechanisms. The conduction of sensory input into the brain is blocked by stimulation of fibres, which descend from the brain stem regions to the dorsal horn and resultantly cause the secretion of neurotransmitters. The patients with fibromyalgia have pain inhibitory system that can be altered in response to the deficiency of the central nervous system. These patients have low concentration of serotonin, dopamine and norepinephrine.[3] Fibromyalgia is also believed to be a stress related disorder, which includes impaired functioning in the hypothalamic pituitary adrenal axis. It is also linked with failure to reduce cortisol concentrations. It has been reported that the patients with fibromyalgia have increased concentration of cortisol as compared to control individuals.[4] In addition, these patients have disturbances in hypothalamic pituitary axis functioning, such as increased levels of cortisol and blunted stress response to ovine corticotrophin releasing hormone. Aberrations in the

activities of autonomic nervous system are recorded in these patients. These abnormalities may include various clinical problems and increased pain perception linked with fibromyalgia through modification of physiological processes, which are required for stress management and pain suppression by decreased synthesis of insulin like growth factor and growth hormone.[4]

The alternations in the autonomic nervous system activity consist of orthostatic hypotension and micro-circulatory hypotension. The patients have low microcirculatory responses to auditory activation and blunt vasoconstriction activity due to cold presser tasks as compared to control individuals.[5] In addition, genetic predisposition is one of the important factors for the progression of this disease. There are various types of genes, but the most important one is linked with neurotransmitters. The transporters of serotonin gene maybe distinguished by single nucleotide polymorphism and considered more frequent in the patients affected by fibromyalgia and psychological stress.[6] Other genes are involved are HLA region, Catechol-O-methyl-transferase gene and dopamine D4 receptor gene in the disease progression. Fibromyalgia is more common in those patients who are influenced by autoimmune diseases.[7] Maintaining the intensity of pain, increased physical activities and restoration of sleep/wake cycle have great significance in reducing the events of fibromyalgia.[8] The treatment and identification of all pain sources which are present in fibromyalgia are fundamental for the proper clinical management. The aim of the present study was to determine the level of various inflammatory markers in patients with fibromyalgia as compared to control individuals.

MATERIAL AND METHODS

A total of 50 patients of fibromyalgia diagnosed in the Department of Orthopaedics (Jinnah Hospital, Allama Iqbal Medical Collage, Lahore, Pakistan) and 50 healthy individuals were included in the study. None of the control individuals were on using multivitamins, cigarette or alcohol, nor did they have depression, liver cancer, diabetes mellitus or malnutrition syndrome. Research Ethical Committee of The Institute of Molecular Biology and Biotechnology approved the research protocol. Five ml of venous blood sample from each participant was taken from the anti-cubital vein. The sample tube was centrifuged within one hour of sample collection and serum was separated for storage at -70 °C until assayed.

Complete blood count of the participants was performed on automated haematology blood analyser (Sysmex version XP-2100). The levels of IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were measured using commercially available kits (Glory Science Human Eliza Kits). MMP-2, 7, 9 were analysed with human diagnostic ELISA kit assay (Bio Compare).

The data was represented as mean and standard deviation. Independent sample *t*-test was applied to compare the data sets and $p<0.05$ was considered as statistically significant.

RESULTS

The data represented in Table-1 summarized the role of inflammatory markers in the patients with fibromyalgia. The mean values of IL-1, IL-2, IL-6, IL-7 and IL-9 were significantly increased in the patients with fibromyalgia when compared to control individuals. Elevated trends of IL-8, IL-17, IL-18, IL-1β and IL-1-RA were recorded in the patients with fibromyalgia as compared to control individuals respectively. The mean values of MMP-2, MMP-7 and MMP-9 were significantly increased in the patients with fibromyalgia as compared to control individuals.

Table-1: Interleukins and matrix metalloproteinases profile in fibromyalgia

VARIABLES	Control (n=50)	Subjects (n=50)	*p*
Interleukin-1 (pg/ml)	0.795±0.009	4.26±1.09	0.001
Interleukin-2 (pg/ml)	35.29±4.29	81.59±4.28	0.026
Interleukin-6 (pg/ml)	10.29±3.29	19.35±2.89	0.018
Interleukin-7 (pg/ml)	14.59±3.29	21.59±3.99	0.037
Interleukin-8 (pg/ml)	19.68±2.88	23.59±4.59	0.024
Interleukin-9 (pg/ml)	42.59±8.77	83.29±7.25	0.013
Interleukin-17 (pg/ml)	55.29±7.59	107.59±11.59	0.041
Interleukin-18 (pg/ml)	49.58±5.88	61.59±8.59	0.000
Interleukin-1β (pg/ml)	0.745±0.018	1.089±0.0095	0.032
Interleukin-1-RA (pg/ml)	3.290±0.956	4.59±1.113	0.015
Matrix metalloproteinases-2 (ng/ml)	423.25±10.59	625.32±18.59	0.038
Matrix metalloproteinases-7 (ng/ml)	49.58±4.28	85.59±10.25	0.000
Matrix metalloproteinases-9 (ng/ml)	41.59±8.59	101.25±10.11	0.011

DISCUSSION

The pro-inflammatory cytokines are considered to be the product of lymphocytes, which are stimulated not only by injury but also by neurons or glial cells. There are various stimuli that can induce this signalling cascade including TNF-α, IL-1, IL-18, IL-8, IL-2, IL-6, IL-17, IL-9, IL-6 and IL-1β. These pro-inflammatory cytokines act synergically and serve to mediate the formation of adhesion molecules through endothelial cells that are crucial for neutrophil recruitment at the site of inflammation. In the peripheral nerves[9,10], IL-1 alone or in combination with TNF-α enhances prostaglandin formation and stimulates substance P expression that reduces pain threshold. Patients with increased level of IL-1 may develop arthralgia, myalgia, headache and fever which can diminished by intake of COX inhibitors.[9]

Some authors observed IL-1-RA expression to be higher in patients with fibromyalgia and no difference was found between the concentration of IL-1 or IL-1-Rβ in these patients.[10] Higher expressions of TNF has been associated with rapid eye movement during sleep in these patients. In the present study, the

increased concentration of IL-8 was found in patients with fibromyalgia as compared to control individuals. This study is also consistent with the research work of Uceyler *et al*[11], who explained the role of IL-8 expressed in the plasma and serum of patients with fibromyalgia. The segregation of IL-8 is regulated by substance, which may lead to degranulation of tissue damage and neutrophil trafficking across vascular wall. In addition, the higher expressions of IL-8 induce sympathetic pain. In fibromyalgia, the upregulation of glial cells may be the consequences of intrathecal increase of cytokines and chemokines.[11] IL-8 levels were also overexpressed in cerebrospinal fluid, which was supported by glial cell activation by sympathetic activity.[11]

IL-6 is one of the most significant mediators for the stimulation of acute phase protein formation and secrete by hepatocytes during pain perception. IL-6 can be linked with depression stress, sympathetic nervous system (SNS) activation, fatigue and hyperalgesia. It is released by endothelial cells, glial cells and neurons. One of the most significant role of IL-6 in food intake, body weight and neurogenesis. Overexpression of IL-6 at the site of tender joints in response to the pain threshold is a significant characteristic for the progression of fibromyalgia.[12]

Aberrant expressions of CD4+ and Th17 have been associated with inflammatory and autoimmune diseases including lupus, psoriasis and rheumatoid arthritis. IL-17 is considered to be major cytokine secreted by Th17 cells and has significant role in the development of autoimmune and inflammatory diseases. The study of Pernambuoco *et al*[13] suggested the increased expression of IL-17 that has strong correlation with IL-10, IL-4, IL-2, IL-1, and IL-6 in these patients. IL-2 level has been associated to enhance cognitive impairment, myalgia, arthralgia and fatigue.[14]

The higher expressions of interleukins are controlled by various intracellular signalling pathways, from which IL-6 includes IL-6 receptor and gp-130 protein. IL-6 attaches to its receptor and signalling mechanism is triggered by suppressing Janus Kinases Signal and Activator of transcription (JAK/STAT) triggered transcription of TNF-α.[15] Some authors suggested that white adipose tissue stimulate the synthesis of IL-6 that cause low grade inflammation in the patients with fibromyalgia.

The patients with IL-2 LAK cell therapy for melanoma or renal cell carcinoma progress tender joints, cognitive impairment, arthralgias and myalgia. The fibromyalgia like symptoms appeared in those patients who were receiving interferon for chronic hepatitis. Brain cells have cytokine receptors and lymphocytes have opiate receptors that combine with substance P and mediate intracellular signalling cascade in these patients.[16] Neuro-immune cytokines have also been observed in these patients.

In the present study, the pro-inflammatory cytokines including IL-8, IL-1RA, IL-5, IL-2, IL-1 and IL-18 are considered to be important for inflammation, SNS, and hypothalamic pituitary axis. The SPS and HPA stimulation can also be facilitated by serotonin and acetylcholine that can be inhibited by opiates, γ-amino butyric acid, IL-2 and IL-6. In fibromyalgia, the sympathetic nervous system innervates immune cells including T-lymphocytes.[17] In addition, beta-agonist can reduce lymphocyte proliferative activity and natural killer cell response. IL-6 and IL-1 can separate beta-adrenergic receptors, IL-8 is stimulator of sympathetic pain and IL-6 can regulate sympathetic nervous system. Interestingly, IL-1 is synthesized by liver induced hyperalgesia through vagal afferents that can be inhibited via administration of IL-RA. In addition, it activates substance P gene expression and is strongly linked with anti-nociception and fever. In fibromyalgia, substance P regulates IL-8 expression that can be inhibited by IL-1RA. However, IL-8 is a pro-inflammatory cytokine that causes neutrophil trafficking around the vascular wall.[13]

TNF-α induces allodynia, pain inducing excitatory amino acids, rapid eye movement, and modulates substance P expression. In healthy individuals, IL-6 produce pain and fatigue, reduced cognitive activity, associates with depression, affects the hyperalgesia of corticosteroid and facilitates T-cell as well as B-cell proliferation. Substance P levels were overexpressed in the patients with fibromyalgia, which trigger the secretion of IL-6 levels.[18] IL-10 enhances B-cell concentrations, endorse energy through down-regulating type 1 activity and reduces TNF-α and IL-6 synthesis via monocytes. The psychological stress in patients with fibromyalgia is regulated through the synthesis of IL-6, IL-10, IL-1-RA and TNF-α. The patients with fibromyalgia have increased levels of IL-1-RA and gp-130 receptor as compared to control individuals.

Matrix Metalloproteinases (MMPs) are zinc dependent endopeptidases which damage extracellular matrix (ECM). MMPs are significantly involved in the plasticity, regeneration and development of central nervous system in the patients with fibromyalgia. In these patients, MMPs are abnormally expressed, cause damage to blood brain barrier, neuronal cell death, demyelination and infiltration of peripheral immune cells.[19] Expression of MMP-2 was higher in activated fibromyalgia and promotes intracellular signalling cascade including NF-ƘB, AP-1 and MAPK in these patients. MMP-8 serves to mediate inflammatory mechanism and expressed in various cell types at the site of inflammation including macrophages, epithelial choroid plexus, granulocytes, plasma cells and neutrophils.[20] The neuroinflammatory response of MMP-9 are strongly linked with TNF-α, which in turn cause tissue damage including diabetes, stroke,

ulcerative colitis, rheumatoid arthritis and fibromylagia.[21]

CONCLUSION

The present study concludes that the levels of inflammatory markers such as IL-1, IL-2, IL-6, IL-7, IL-8, IL-9, IL-17, IL-18, IL-1β and IL-1-RA were significantly higher among the fibromyalgia patients as compared to controls. It signifies the said inflammatory markers may have prominent role in the development and progression of disease condition.

ACKNOWLEDGEMENT

The authors acknowledge the contribution of all students of Lab-313 and the respected leadership of the Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.

REFERENCES

1. Bennett RM, Jones J, Turk DJ, Russell IJ, Matallana L. An internet survey of 2,596 people with fibromyalgia. BMC Musculoskeletal Disord 2007;8:27.
2. Bellato E, Marini E, Castoldi F, Barbasetti N, Mattei L, Bonasia DE, *et al*. Fibromyalgia syndrome: etiology, pathogenesis, diagnosis, and treatment. Pain Res Treat 2012;2012:246130.
3. Cohen H, Buskila D, Neumann L, Ebstein RP. Confirmation of an association between fibromyalgia and serotonin transporter promoter region (5-HTTLPR) polymorphism, and relationship to anxiety-related personality traits. Arthritis Rheum 2002;46(3):845–7.
4. Schaefer C, Mann R, Masters ET, Cappelleri JC, Daniel SR, Zlateva G, *et al.* The comparative burden of chronic widespread pain and fibromyalgia in the United States. Pain Pract 2016;16(5):565–79.
5. Grayston R, Czanner G, Elhadd K, Goebel A, Frank B, Üçeyler N, *et al.* A systematic review and meta-analysis of the prevalence of small fiber pathology in fibromyalgia: Implications for a new paradigm in fibromyalgia etiopathogenesis. Semin Arthritis Rheum 2019;48(5):933–40.
6. Russell IJ, Vaeroy H, Javors M, Nyberg F. Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. Arthritis Rheum 1992;35:550–6.
7. Aaron LA, Buchwald D. Chronic diffuse musculoskeletal pain, fibromyalgia and co-morbid unexplained clinical conditions. Best Pract Res Clin Rheumatol 2003;17:563–74.
8. Arnold LM, Hudson JI, Keck PE, Auchenbach MB, Javaras KN, Hess EV. Comorbidity of fibromyalgia and psychiatric disorders. J Clin Psychiatry 2006;67:1219–25.
9. Geiss A, Rohleder N, Anton F. Evidence for an association between an enhanced reactivity of interleukin-6 levels and reduced glucocorticoid sensitivity in patients with fibromyalgia. Psychoneuroendocrinology 2012;37:671–84.
10. Borchers AT, Gershwin ME. Fibromyalgia: a critical and comprehensive review. Clin Rev Allergy Immunol 2015;49(2):100–51.
11. Uceyler N, Häuser W, Sommer C. Systematic review with meta-analysis: cytokines in fibromyalgia syndrome. BMC Musculoskelet Disord 2011;12:245.
12. Jarlborg M, Gabay C. Systemic effects of IL-6 blockade in rheumatoid arthritis beyond the joints. Cytokine 2022;149:155742.
13. Pernambuco AP, Schetino LP, Alvim CC, Murad CM, Viana RS, Carvalho LS, *et al.* Increased levels of IL-17A in patients with fibromyalgia. Clin Exp Rheumatol 2013;31(6 Suppl 79):S60–3.
14. Bazzichi L, Giacomelli C, De Feo F, Giuliano T, Rossi A, Doveri M, *et al*. Antipolymer antibody in Italian fibromyalgic patients. Arthritis Res Ther 2007;9(5):R86.
15. Janik JE, Miller LL, Longo DL, Powers GC, Urba WJ, Kopp WC, *et al.* Phase II trial of interleukin 1 alpha and indomethacin in treatment of metastatic melanoma. J Natl Cancer Inst 1996;88(1):44–9.
16. Malcangio M, Bowery NG, Flower RJ, Perretti M. Effect of interleukin-1 beta on the release of substance P from rat isolated spinal cord. Eur J Pharmacol 1996;299:113–8.
17. Carbia-Nagashima A, Arzt E. Intracellular proteins and mechanisms involved in the control of gp130/JAK/STAT cytokine signaling. IUBMB Life 2004;56(2):83–8.
18. Wallace DJ, Linker-Israeli M, Hallegua D, Silverman S, Silver D, Weisman MH. Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study. Rheumatology (Oxford) 2001;40:743–9.
19. Bellinger DL, Lorton D. Autonomic regulation of cellular immune function. Auton Neurosci 2014;182:15–41.
20. Schwarz YA, Amin RS, Stark JM, Trapnell BC, Wilmott RW. Interleukin-1 receptor antagonist inhibits interleukin-8 expression in A549 respiratory epithelial cell infected *in vitro* with a replication-deficient recombinant adenovirus vector. Am J Respir Cell Mol Biol 1999:21:388–94.
21. Hansmann F, Herder V, Kalkuhl A, Haist V, Zhang N, Schaudien D, *et al.* Matrix metalloproteinase-12 deficiency ameliorates the clinical course and demyelination in Theiler's murine encephalomyelitis. Acta Neuropathol 2012;124:127–42.

Address for Correspondence:

Dr. Arif Malik, Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan. **Cell:** +92-321-8448196

Email: arifuaf@yahoo.com

Received: 11 Jan 2022 **Reviewed:** 23 Feb 2022 **Accepted:** 23 Feb 2022

Contribution of Authors:

NT: Writing, Reviewing
HS: Reviewing, Editing
RZB: Critical analysis, Reviewing
FZB: Writing, Analysing
SW: Methodology, Analysis, Writing
AM: Conceptualization, Conclusion

Conflict of interest: Authors declare no conflict of interest
Funding: None to declare