Increased Level of circulatory cytokines and stress markers to develop fibromyalgia: A condition that causes pain

Nusrat Tariq[1], Muhammad Rafiq[1,] Tahir Maqbool*[1], Inam Ullah[1], Shabana Akhtar[1], Sadia Naz[2], Imtiaz Mustafa[1], Arif Malik[1] and Qurban Ali[*3]

1. Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore Pakistan
2. Allied Health Sciences, The University of Lahore, Lahore, Pakistan

3. Department of Plant Breeding and Genetics, The university of Punjab Lahore, Lahore Pakistan

Correspondence: Tahir Maqbool, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.

Abstract
Fibromyalgia is a chronic, extensive musculoskeletal pain and associated fatigue, sleep disturbance, and other cognitive and physical symptoms. Studies have indicated that different cytokines and chemokine's play an important role in inducing symptoms like pain, fatigue, fibromyalgia, etc. The primary objective of the current study was to stipulate the role of up regulated cytokines in the development of fibromyalgia. A total of 300 participants were recruited in this study divided into 150 healthy control and 150 diseased groups. After getting an informed consent, 5ml of blood was drawn into specialized vials for the analysis of various markers. For the quantification of different cytokines, the enzyme linked immunosorbent assay was used. Levels of lipid peroxidation products such as MDA, IsoP, 8-OHdG, 4-HNE followed by the cytokines remained significantly higher among the patients when compared to healthy individuals. Levels of IL-1, 2, 4, 6, 8, 13 and 17 remained significantly higher among the group of Fibromyalgia patients Findings of the study stipulate the role of elevated levels of cytokines and other stress markers in the development and progression of the disease condition among the subject group. It states how elevated levels of interleukins can significantly increase the chances of developing FM conditions among patients. Therefore, the interleukins may serve as potential predictors to down regulate the progression of fibromyalgia.
Keywords: Fibromyalgia, Cytokines, MDA, IsoP, 8-OHdG, 4-HNE

1.0 Introduction

Fibromyalgia (FM), is a chronic disorder that is characterized by widespread pain, stiffness of joints, fatigue, insomnia, cognitive dysfunction, and mood change without any fundamental organic disease (Tekin*et al*., 2013). Nearly, overall 5% of the population is affected worldwide, which indicates the incidence of rheumatic diseases is much more common in females compared to that in males (Pernambuco*et al*., 2016). Fibromyalgia remains a disorder that is not completely understood yet. Pathophysiological and etiological factors of the disease are not well explained. Still, many studies are being conducted to thoroughly explain the fundamental pathophysiological mechanism of fibromyalgia, which is unknown. It has been proposed that symptoms of fibromyalgia originate after the interactions within the hypothalamus-pituitary-adrenal (HPA) axis, immune system, and autonomic nervous system (Ang*et al*., 201). Fibromyalgia is assumed as a non-inflammatory disease, however, a recent study believed that cytokines have a role in the etiology of fibromyalgia by Wallace in 1988 (Wallace*et al*., 1999).

When there is an imbalance between inflammatory and anti-inflammatory cytokines that ultimately leads to increase inflammation that further leads to elevated pain intensity among fibromyalgia patients. While many studies have executed the role of elevated cytokines among patients with fibromyalgia (Uçeyler*et al*., 2011), Also, it has been found a communication among the nervous and immune systems via cytokines (Staud*et al*., 2015). In addition, literature signifies the relationship of the said inflammatory cytokines and stress markers such as IL-1, 1 β, 6, 8, and TNF-α with the incidence of fibromyalgia and its related symptoms that include, fatigue, cognitive dysfunctions, hyperalgesia, stress, sleep disorders, and anxiety (Bazzichi*et al*., 2007; Rodriguez*et al*., 2014; Xiao*et al*., 2013). Still, how the functioning of the central nervous system is affected by cytokines and how they play their role in developing widespread pain in fibromyalgia, are the questions, which are still to be answered. In recent studies, there are some evidance that suggested that in the pathophysiology of fibromyalgia, oxidative stress might play its role (Wallace*et al*., 1999; Ranzolin*et al.*, 2016). In fibromyalgia patients, oxidative stress levels were significantly increased as compared to healthy individuals (Chung*et al*., 2009; Ozgocmen*et al*., 2006).

One oxidative stress marker oxidized low-density lipoprotein (Ox-LDL) identifies a link between abnormal inflammation and the immune system (Tekin*et al*., 2013). One of the characteristics of this lipoprotein is pro-inflammation which is responsible for an increase in the production of inflammatory cytokines as well as an increase in cell adhesion, proliferation, migration, and apoptosis (Tekin*et al*., 2013; Greig*et al*., 2012). In certain diseases, there is an increase due to circulating oxidized low-density lipoproteins, which are related to increased inflammation and oxidative stress (Shen*et al*., 2013). These findings have opened the path to finding the cytokines expression in fibromyalgia. Thus, the current study was designed to assess the difference in serum levels of lipid peroxidation products, and interleukins IL-1β, TNF-α, IL-8, among patients with fibromyalgia from the local population when compared with healthy individuals and to determine the prognostic importance of said biochemical variables.

2.0 Materials and methods

StudyAims

The present study was designed to investigate the role of lipid peroxidation products (MDA, IsoP, 8-OHdG, 4-HNE) and interleukins (ILs) in the development of fibromyalgia. To maintain a good quality of life in both males and females of fibromyalgia.

StudyParticipants

All the patients (300) were screened at the Jinnah hospital Lahore. Informed consent was obtained before being included in this study. A total of 300 patients wereenrolled in the study divided into 150 diagnosed with fibromyalgia group, 150 healthy individuals as a control group.

Sampling Technique

Convenient non-probability sampling technique was employed to select the study participants.

Inclusionandexclusioncriteria

Participants who accepted to take part in the study were recruited. Selected subjects were between 30-50 years. They were further classified into two groups. 150 controls, and 150 with fibromyalgia. All people affected with other diseases or under medication that can affect the study were excluded. The control group was made up of healthy volunteers present either in the institute or in the family. Moreover, none of the control individuals were included with a history of chronic infections and metabolic dysfunction such as hypertension, diabetes, and cancer. None of the control subjects was taking any medication.

Evaluation of CBC (complete blood count)

Complete blood count of the selected subjects was performed on the automated hematology blood analyzer by Sysmex (version. XP-2100).

Estimation of thiobarbituric acid reactive substances (tbars)/ malondialdehyde (MDA)

Levels of Malondialdehyde (MDA) were determined by (Ghazal *et al.,* 2022) with the help of a calorimetric method that includes measurement of Thiobarbituric acid reactive substances (TBARS). For the estimation of MDA about 0.2ml of the sample was drawn and was later subjected to the SDS, TBA, and acetic acid. After centrifuging the samples at RPM of 3000 for about ten-minute the supernatant was separated and the observation was read at 532nm using a spectrophotometer. Finally, the measured qualitative values were quantified by drawing a standard curve and the levels were expressed in units (TBARS)/g.

Estimation of human-8-iso-pgf2*α*, 4HNE, OHDG

The levels of isoprostanes, 4HNE, OHDG were determined by using maqbool t (30) protocol briefly 100 samples loaded toeach well for 90 min at 37℃, after the sampe was removed from each well 100µL of Biotinylated Detection Ab loaded to each well for 1 hour at 37°C. after that each well was washed three times with wash buffer. Then 100 µL of HRP Conjugate working solution was added to each well for 30 min at 37°C. Again wased five times and 90 µL of Substrate Reagent was added each well for about 15 min at 37°C. Finally 50 µL of Stop Solution was added to each well and the optical density (OD value) of each well was obtained with a micro-plate reader set to 450 nm.

Estimation of cytokines (ILs-1, 2, 8, 6, 17, 13, 4 &TNFa):

The levels of ILs and TNF-α were determined by the human-available diagnostic ELISA kitmethod. The standard was prepared from 200pg/ml and assessable concentration of interleukinsand TNF-α remained at 3 pg/mL. First of all, 100µL of serum sample was added to the ELISAplate and incubated at room temperature for 120 minutes. After incubation, the plate was washedwith washing buffer solution. After the removal of extra water from the ELISA plate, the platewas inverted on a paper towel. 100µl of HRP conjugate solution was added into each well andincubated at room temperature for 1 hour. The plate was washed again and dried on a paper towelfor the removal of residual water. After that, the substrate was added into each well with aconcentration of 100µL and kept in dark room temperature for incubation for 15minutes. Later on, TMB was added with the amount of 100µl into each well and placed for onehour. In the last, 50µl of stop solution was added which provided the color perception during this reaction,whichshowedthepresenceofTNF-αandinterleukinsintheserumsampleofpatientswith diabetic nephropathy. Finally, the absorbance was taken at the 460 nm wavelength byELISA reader.

Statistical Analysis

All data of experimental groups were expressed as mean± SEM. For statistical analysis, group means were compared by t-test to identify differences between groups by using a graph pad prism. A p-value less than 0.05 was considered significant from statistical analysis.

3.0 Results

The Current study was designed to investigate the role of inflammatory cytokines, oxidative stress and cellular stress response in patients suffering from fibromyalgia compared to healthy subjects. The total number of individuals recruited into the study were 300, including 150 patients with fibromyalgia, , and 150 normal healthy controls. All the participants inthe current studyweremachedforage,sexand bodymassIndex[BMI].The demographic profile of the patients andcontrols is summarized in Table 1 and Figure1.

Table 1. Demographic profile of fibromyalgia..Subject's verses healthy age matched control

VARIABLES	CONTROL (n=150)	SUBJECT (n=150)	*P*- VALUE
Weight (kg)	68.00±3.00	69.00±3.00	0.7040
Age (yrs)	61.00±3.606	62.33±3.512	0.6702
BMI (kg/m)2	24.67±4.163	26.08±2.881	0.6540
Systolic BP (mmHg)	121.0±3.606	122.7±3.215	0.5823
Diastolic BP (mmHg)	81.3606±	82.67±4.041	0.6223

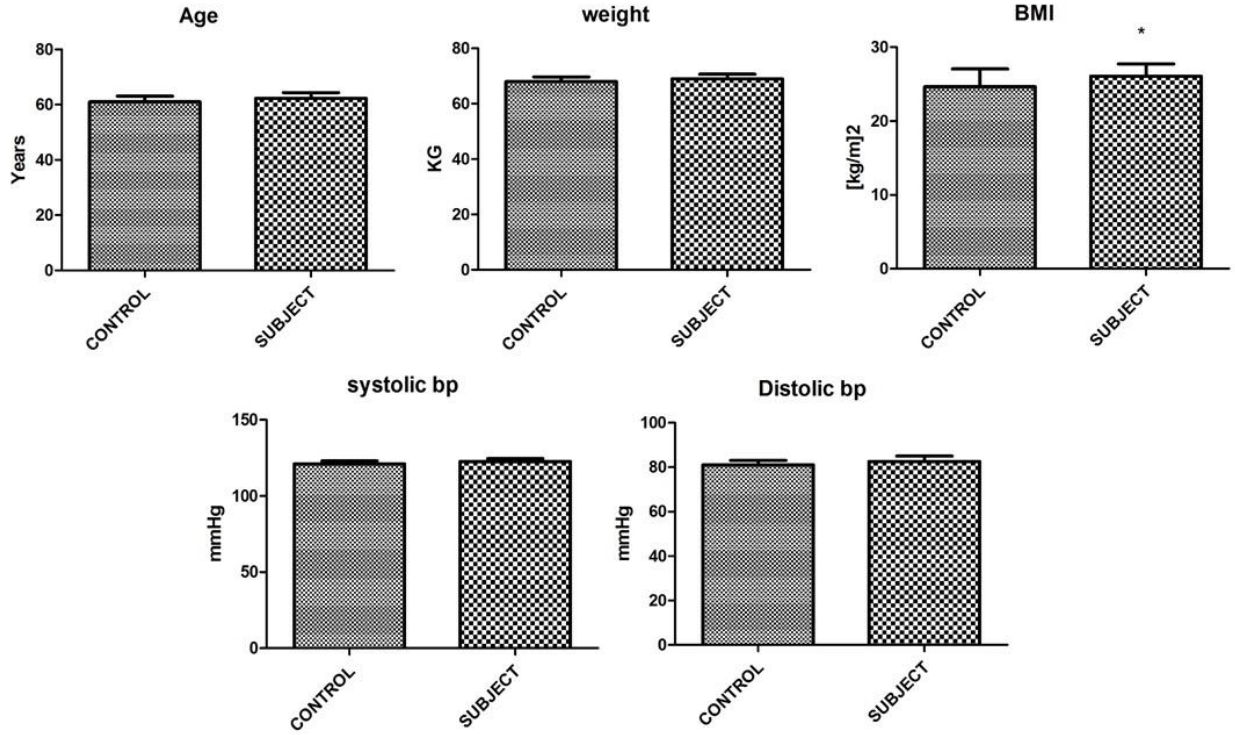

Fig 1 is showing the demographic data of fibromyalgia patients and healthy control. Graph containing Age, Weight, BMI, Systolic and diastolic blood pressure. There is no significant difference between the groups.

Further biochemical parameters were performed where a decreased levels of Hb, RBCs and HCT while increased levels of WBCs and platelets, were observed. Biochemical parameters involve in current study are in table 2, Figure 2.

TABLE 2: Profile of different biochemical parameters in fibromyalgia vs healthy control

VARIABLES	CONTROL (n=150)	SUBJECT (n=150)	*P*- VALUE
HB	14.30±0.8597	10.23±0.4509	0.0019
RBCs	5.900±2.095	4.090±0.2594	0.0059
HCT	45.15±1.975	20.20±1.513	0.0001
WBCs	5.400±0.7550	9.317±0.7147	0.028
Platelets	225.7±6.028	268.1±15.95	0.0125

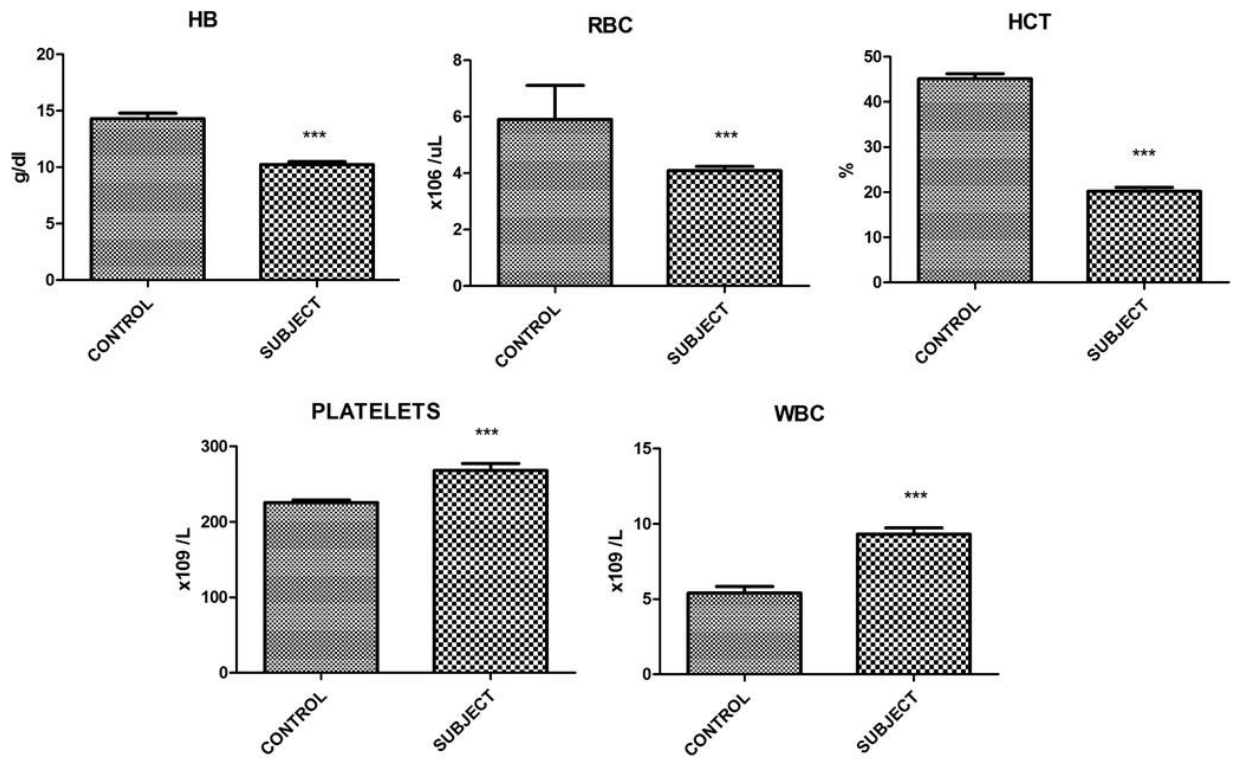

Figure 2 Showing the biochemical parameters of fibromyalgia patients compared to healthy control. Graph showing HB, RBCs, HCT, Platelets, and WBCs. Where *** showing the significant difference between the groups. P≤0.05

The levels of stress markers differed significantly in fibromyalgia. Patients were screened for the levels of MDA, Isoprostanes, 4-HNE, and 8-OHdG. Levels of malondialdehyde (MDA) were significantly increased in the subjects as compared to health controls. Likewise levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-hydroxynonenal (4-HNE) and Isoprostanes were increased in the group of fibromyalgia compared to health control as shown in table3, and figure 3).

Table- 03:Profile of different Variables Having Potential Role in the development of fibromyalgia

VARIABLES	**CONTROL (n=150)**	**SUBJECT (n=150)**	***P*- VALUE**
MDA (nmol/ml)	0.95±0.13	4.19±0.25	0.015
Isoprostanes (pg/ml)	31.09±5.87	88.26±7.19	0.011
8-OHdG (pg/ml)	0.11±0.03	1.08±0.047	0.036
4-HNE (µmol/ml)	1.99±0.35	10.26±0.68	0.041

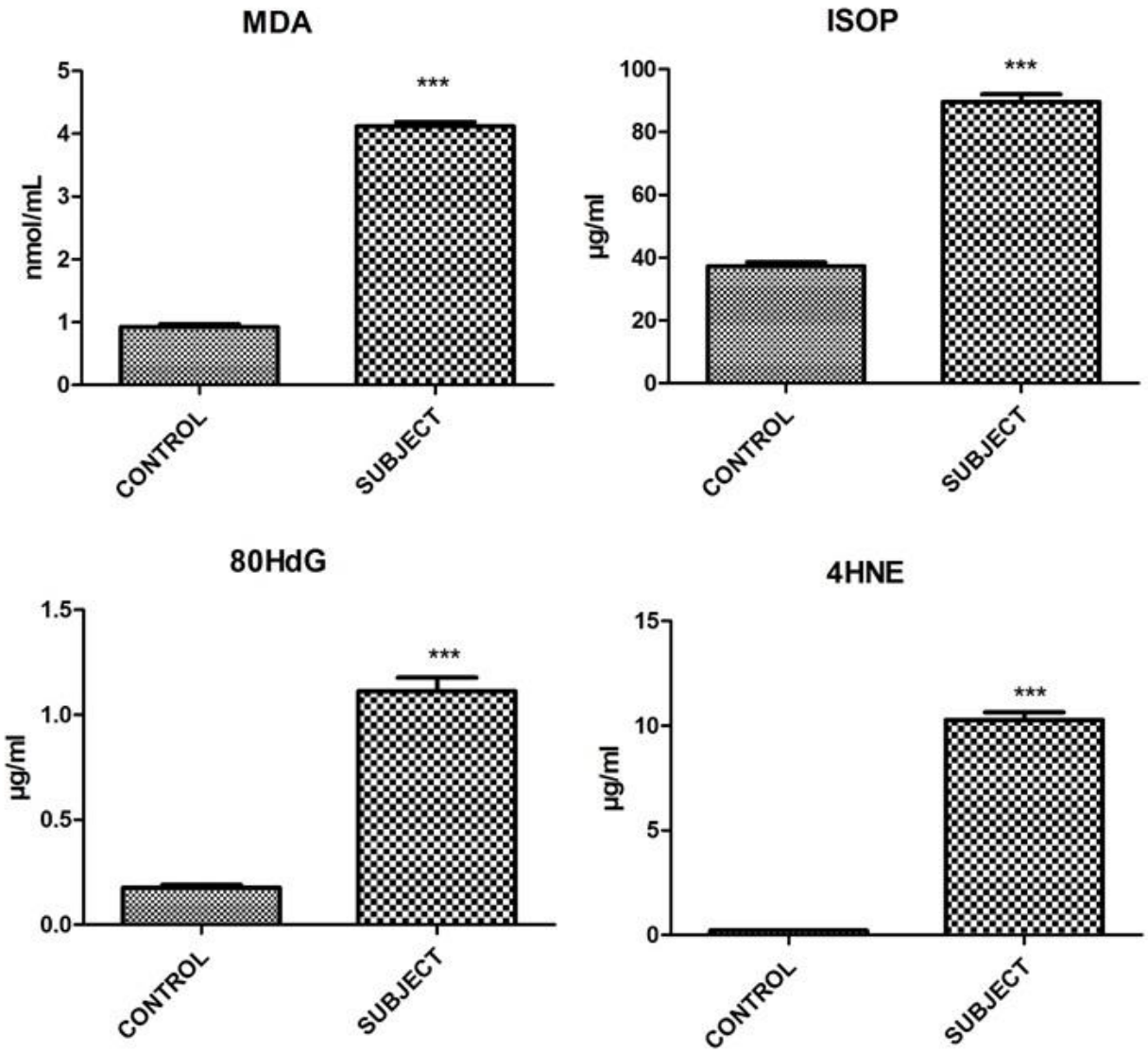

Figure 3 showing the cytokines of fibromyalgia patients compared to healthy control. Graph showing MDA, ISOP, 8OHdG and 4HNE. Where *** represents the significant difference between the groups. P≤0.05.

All the subject (fibromyalgia) group represented significantly increased levels of interleukins. Levels of IL-1, 2, 4, 6, 8, 13 and 17 remained significantly increased in the group of fibromyalgia (6.19±0.59, 5.10±0.29, 6.08±0.42, 5.23±0.45, 7.19±0.68, 8.16±0.67, 8.11±0.67 pg/ml, p value= 0.000, 0.019, 0.008, 0.014, 0.039, 0.016 and 0.017) as compared to controls (2.09±0.25, 4.19±0.21, 4.01±0.38, 3.11±0.38, 3.14±0.31, 3.11±0.16 and 5.19±0.58 pg/ml) respectively as shown in table 4 and figure 4.

Table- 04: Profile of different variables having potential role in the development of fibromyalgia

VARIABLES	CONTROL (n=150)	SUBJECT (n=150)	*P*- VALUE
IL-1 (pg/ml)	2.09±0.25	6.19±0.59	0.000
IL-2 (pg/ml)	4.19±0.21	5.10±0.29	0.019

IL-8 (pg/ml)	3.14±0.31	7.19±0.68	0.039
IL-6 (pg/ml)	3.11±0.38	5.23±0.45	0.014
IL-17 (pg/ml)	5.19±0.58	8.11±0.67	0.017
IL-13 (pg/ml)	3.11±0.16	8.16±0.67	0.016
IL-4 (pg/ml)	4.01±0.38	6.08±0.42	0.008
TNF-α (pg/ml)	10.99±1.22	21.58±1.56	0.019

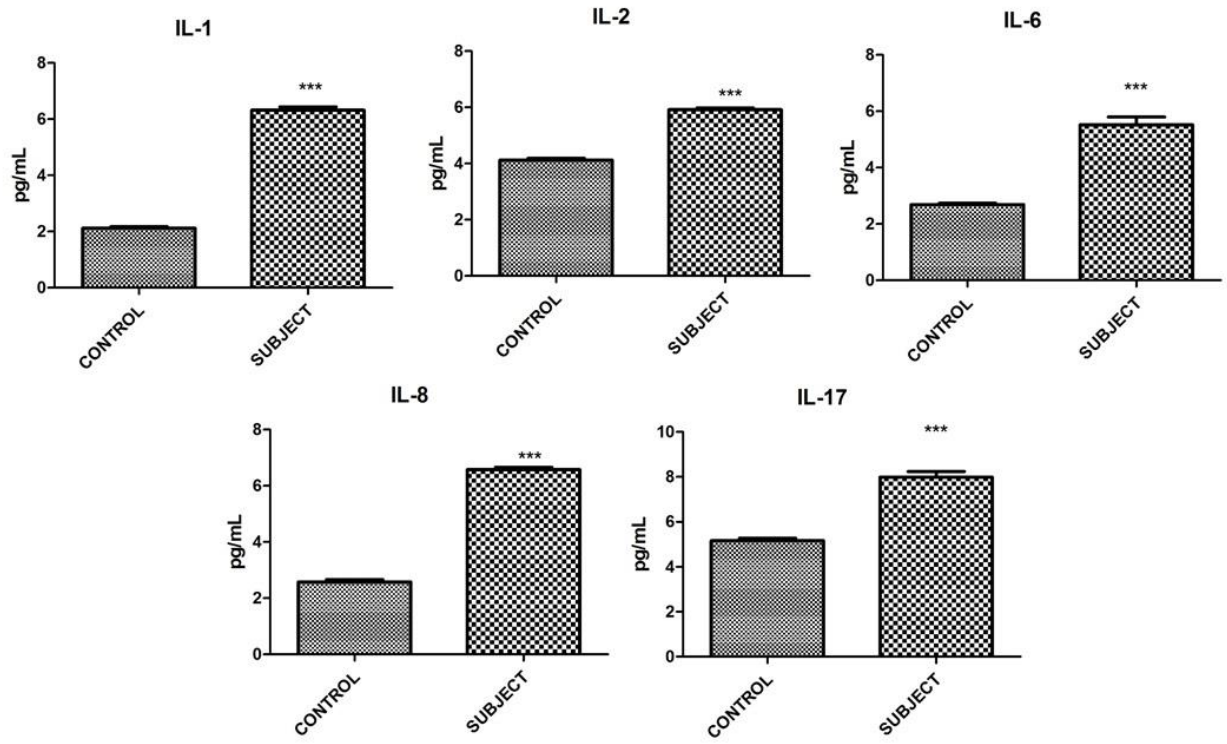

Figure 4 Showing the level of interleukins in fibromyalgia patients compared to healthy control. Graph showing IL-1, 2, 6, 8 and 17. Where *** showing the significant difference between the groups. P≤0.05.

4.0 Discussion

In this study, pro-inflammatory serum cytokines and levels of oxidative stress biomarkers were estimated and evaluated among the patients with fibromyalgia by comparing the results of patients with healthy individuals. Similarly, another fact was observed that executes statistically significant differences among the levels of said cytokines such as TNF-α, ILs, and other oxidative stress biomarkers among fibromyalgia patients as compared to the control group. Increased levels of such cytokines are often directly related to the severity of pain index among patients of fibromyalgia. In the literature, there seems to be controversy in the studies investigating levels of serum cytokines in fibromyalgia patients. Some authors have raised their statements in agreeing with the said facts whereas, there are some opposing statements available in the literature. They described how the increase in levels of said cytokines is related to the increased incidence of fibromyalgia among the patients (Bazzichi*et al.,* 2007; Gur*et al*., 2002; Kadetoff*et al.,* 2012 7,20-Uçeyler*et al.,* 2006). Moreover, the literature also depicted the role of elevated levels of said cytokines may play a significant role in the development of symptoms that lead to the progression of disease conditions among the subjects (Uçeyler*et al.,* 2006). Literature

signifies how cytokines such as IL-1, 6, 8, and TNF-α are responsible for the development of peripheral nephropathy, other disorders of the sympathetic nervous system, and brain chemicals i.e., catecholamines that have a direct role in the release of various cytokines (Gür *et al.,* 2002). The IL-8 induces hyperalgesia, and activated the sympathetic nervous system through a mechanism that is independent of prostaglandins (Dinarello et al. 1998). In addition, activation of glial cells in chronic pain, such as fibromyalgia, leads to hyperalgesia and central sensitization (Hernandez et al., 2010). Activation of glial cells triggers the release of cytokines such as TNF-α, IL-1β, and IL-8. Few studies indicate that in fibromyalgia the level of IL-8 is not significant as compared to healthy individuals (Bradley, 2010) while some studies have shown an increase in levels in fibromyalgia patients (Bazzichi*et al.,* 2007; Kadetoff*et al.,* 2012). Gur*et al.* in the said study depicted the role of interleukine-8 (IL-8) in the pain severity index among the patients with fibromyalgia. Kadetoff*et al.* also reported similar findings which he carried out among the fifteen fibromyalgia patients that executed how the increase in IL-8 levels in the cerebrospinal fluid (CSF) of the subjects was responsible for the hyperalgesia (Kadetoff*et al.,* 2012). Some similar findings are reported in the current study. Although the levels of IL-8 were not significantly different among both groups it executed a statistically significant correlation between the index of VAS scores and IL-8 in the patients of fibromyalgia suggests the significance of IL-8 in the pain severity index of fibromyalgia patients. Thus, it may be considered that such correlation supports the role of IL-8 in hyperalgesia. Not only IL-8. Findings of the current study also relate exogenous and endogenous impacts of IL-1 with the physical anomalies that include conditions such as weight loss, regulation of the immune system, sleep cycle, epilepsy, functions of the nervous system, functions related to the neuronal transmission, and other neurological disorders (Cordero*et al*., 2011).

The findings of the current study also indicate how elevated levels of IL-1 along with elevated levels of IL-8 have a significant role in the induction of hyperalgesia with vagal stimuli. It signifies how the release of substance P, contributes to the development of the conditions related to the fever (Cordero*et al*., 2011). Although, it may be understood that IL-1 poses a direct influence on the symptoms related to fatigue myalgia, depression, hyperalgesia, and somnolence among fibromyalgia patients. Some studies also relate the fact that levels of IL-1β among patients with FM (Kadetoff*et al.,* 2012). Tumor necrosis factor-alpha is produced under the stimuli of any inflammatory cytokines or maybe any other infection. As known the major role of TNF-α is to defend an organism against the infection that arises due to the stimuli of any bacterial, viral, or parasite (Maqbool*et al*., 2019). In addition, synthesis of TNF-α can be observed due to the increased oxidative stress, release of substance P, or any other rapid eye movement (REM) sleep (Rubia*et al.,* 2013). Thus, said evidence relates the role of TNF-α is responsible for hyperalgesia, myalgia, fatigue, depression, and REM sleep in FM patients along with the findings of IL-1. Current study, stipulates no statistical difference in the levels of serum tumor necrosis factor among the group of controls and patients with fibromyalgia (FM). However, the study supports a significant correlation between the levels of IL-6 and serum TNF-α in patients. Keeping in mind the significance of the said study it is the first one to state the correlation between IL-6 and fibromyalgia (Greig*et al.,* 2012;Mansoor*et al.,* 2022). The Present study signifies its pro-inflammatory effects and elevated release of such cytokines that may be responsible for cell adhesion, migration, proliferation, and apoptosis.

5.0 Conclusion

The findings of the study conclude and support the hypothesis that indicates the levels of cytokines and other biochemical variables in the development and progression of fibromyalgia

condition among the patients. It indicates that elevated levels of inflammatory cytokines are directly responsible for the increased incidence of the disease condition and worsening pain intensity among patients of fibromyalgia. Therefore, early determination and monitoring of the said variables could play a significant role in lowering the risks and incidence of complications related to fibromyalgia conditions.

Conflict of interest

The authors declare no conflict of interest

References

1. Mease, P., 2005. Fibromyalgia syndrome: review of clinical presentation, pathogenesis, outcome measures, and treatment. *The Journal of Rheumatology Supplement*, *75*, pp.6-21.
2. Pernambuco, A.P., Schetino, L.L., Carvalho, L.S.C. and Reis, D.A., 2016. Involvement of oxidative stress and nitric oxide in fibromyalgia pathophysiology: a relationship to be elucidated. *Fibromyalgia: Open Access*, *1*(1), p.105.
3. Ang, D.C., Moore, M.N., Hilligoss, J. and Tabbey, R., 2011. MCP-1 and IL-8 as pain biomarkers in fibromyalgia: a pilot study. *Pain Medicine*, *12*(8), pp.1154-1161.
4. WALLACE, D.J., MARGOLIN, K. and WALLER, P., 1988. Fibromyalgia and interleukin-2 therapy for malignancy. *Annals of internal medicine*, *108*(6), pp.909-909.
5. Üçeyler, N., Häuser, W. and Sommer, C., 2011. Systematic review with meta-analysis: cytokines in fibromyalgia syndrome. *BMC musculoskeletal disorders*, *12*(1), pp.1-15.
6. Staud, R., 2015. Cytokine and immune system abnormalities in fibromyalgia and other central sensitivity syndromes. *Current Rheumatology Reviews*, *11*(2), pp.109-115.7.
7. Bazzichi, L., Rossi, A., Massimetti, G., Giannaccini, G., Giuliano, T., De Feo, F., Ciapparelli, A., Dell Osso, L. and Bombardieri, S., 2007. Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clinical and experimental rheumatology*, *25*(2), p.225.
8. Rodriguez, I., Agmon, N., Howard, A., Shoenfeld, Y., 2014. Fibromyalgia and cytokines. ImmunolLett. 16(1), p200–203.
9. Xiao, Y., Haynes, W.L., Michalek, J.E. and Russell, I.J., 2013. Elevated serum high-sensitivity C-reactive protein levels in fibromyalgia syndrome patients correlate with body mass index, interleukin-6, interleukin-8, erythrocyte sedimentation rate. *Rheumatology international*, *33*(5), pp.1259-1264.
10. Ranzolin, A., Duarte, A.L.B.P., Bredemeier, M., da Costa Neto, C.A., Ascoli, B.M., Wollenhaupt-Aguiar, B., Kapczinski, F. and Xavier, R.M., 2016. Evaluation of cytokines, oxidative stress markers and brain-derived neurotrophic factor in patients with fibromyalgia–A controlled cross-sectional study. *Cytokine*, *84*, pp.25-28.
11. Chung, C.P., Titova, D., Oeser, A., Randels, M., Avalos, I., Milne, G.L., Morrow, J.D. and Stein, C.M., 2009. Oxidative stress in fibromyalgia and its relationship to symptoms. *Clinical rheumatology*, *28*(4), pp.435-438.
12. Ozgocmen, S., Ozyurt, H., Sogut, S., Akyol, O., Ardicoglu, O. and Yildizhan, H., 2006. Antioxidant status, lipid peroxidation and nitric oxide in fibromyalgia: etiologic and therapeutic concerns. *Rheumatology international*, *26*(7), pp.598-603.
13. Tekin, I.O., Orem, A. and Shiri-Sverdlov, R., 2013. Oxidized LDL in inflammation: from bench to bedside. *Mediators of Inflammation*, *2013*.
14. Greig, F.H., Kennedy, S. and Spickett, C.M., 2012. Physiological effects of oxidized phospholipids and their cellular signaling mechanisms in inflammation. *Free Radical Biology and Medicine*, *52*(2), pp.266-280.

15. Shen, Y., Yang, T., Guo, S., Li, X.O., Chen, L., Wang, T. and Wen, F., 2013. Increased serum ox-LDL levels correlated with lung function, inflammation, and oxidative stress in COPD. *Mediators of inflammation*, *2013*.
16. Mansoor, G., Tahir, M., Maqbool, T., Abbasi, SQ., Hadi, F., Shakoori,TA., Akhtar, S., Rafiq, M., Ashraf, M., Ullah, I., 2022. Increased Expression of Circulating Stress Markers, Inflammatory Cytokines and Decreased Antioxidant Level in Diabetic Nephropathy. *Medicina*. 58(11):1604.
17. Wang, H., Moser, M., Schiltenwolf, M. and Buchner, M., 2008. Circulating cytokine levels compared to pain in patients with fibromyalgia—a prospective longitudinal study over 6 months. *The Journal of rheumatology*, *35*(7), pp.1366-1370.
18. Mendieta, D., De la Cruz-Aguilera, D.L., Barrera-Villalpando, M.I., Becerril-Villanueva, E., Arreola, R., Hernández-Ferreira, E., Pérez-Tapia, S.M., Pérez-Sánchez, G., Garcés-Alvarez, M.E., Aguirre-Cruz, L. and Velasco-Velázquez, M.A., 2016. IL-8 and IL-6 primarily mediate the inflammatory response in fibromyalgia patients. *Journal of neuroimmunology*, *290*, pp.22-25.
19. Wallace, D.J., Linker-Israeli, M., Hallegua, D., Silverman, S., Silver, D. and Weisman, M.H., 2001. Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study. *Rheumatology*, *40*(7), pp.743-749.
20. Malek, H., Ebadzadeh, M.M., Safabakhsh, R., Razavi, A. and Zaringhalam, J., 2015. Dynamics of the HPA axis and inflammatory cytokines: insights from mathematical modeling. *Computers in Biology and Medicine*, *67*, pp.1-12.
21. Gur, A., Karakoc, M., Erdogan, S., Nas, K., Cevik, R. and Sarac, A.J., 2002. Regional cerebral blood flow and cytokines in young females with fibromyalgia. *Clinical and experimental rheumatology*, *20*(6), pp.753-760.
22. Kadetoff, D., Lampa, J., Westman, M., Andersson, M. and Kosek, E., 2012. Evidence of central inflammation in fibromyalgia—increased cerebrospinal fluid interleukin-8 levels. *Journal of neuroimmunology*, *242*(1-2), pp.33-38.
23. Üçeyler, N., Valenza, R., Stock, M., Schedel, R., Sprotte, G. and Sommer, C., 2006. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *54*(8), pp.2656-2664.
24. Gür, A., Karakoç, M., Nas, K., Denli, A. and Saraç, J., 2002. Cytokines and depression in cases with fibromyalgia. *The Journal of rheumatology*, *29*(2), pp.358-361.
25. Dinarello, C.A.,1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol.* 16. pp.457–499. [PubMed] [Google Scholar].
26. Hernandez, M.E., Becerril, E., Perez, M., Leff, P., Anton, B., Estrada, S., Estrada, I., Sarasa, M., Serrano, E. and Pavon, L., 2010. Proinflammatory cytokine levels in fibromyalgia patients are independent of body mass index. *BMC research notes*, *3*(1), pp.1-5.
27. Bradley, J., 2008. TNF-mediated inflammatory disease. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *214*(2), pp.149-160.
28. Jin, W., Zhao, Y., Yan, W., Cao, L., Zhang, W., Wang, M., Zhang, T., Fu, Q. and Li, Z., 2012. Elevated circulating interleukin-27 in patients with coronary artery disease is associated with dendritic cells, oxidized low-density lipoprotein, and severity of coronary artery stenosis. *Mediators of inflammation*, *2012*.

29. Cordero, M.D., Alcocer, G.E., Cano, F.J.,Miguel, M., Carrión, A.M., Navas, P., 2011. Clinical symptoms in fibromyalgia are better associated to lipid peroxidation levels in blood mononuclear cells rather than in plasma. *PLoS One.* 6 pp.26915–26915
30. La Rubia, M., Rus, A., Molina, F. and Del Moral, M.L., 2013. Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status. *ClinExpRheumatol*, *31*(6 Suppl 79), pp.S121-7.
31. Maqbool, T., Awan, S.J., Malik, S., Hadi, F., Shehzadi, S. and Tariq, K., 2019. In-vitro anti-proliferative, apoptotic and antioxidative activities of medicinal herb Kalonji (Nigella sativa). *Current Pharmaceutical Biotechnology*, *20*(15), pp.1288-1308.
32. Mansoor, G., Muhammad, T., Hadi, F., Maqbool, T., Abbasi, S. Q., Shakoori, T. A. and Malik, A. (2022) "Deranged Haematological Profile and Dyslipidaemiain Diabetes Induced Nephropathy", Journal of Pharmaceutical Research International, 34(29B), pp. 64-72.