Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 2806 - 2818
Received 08 November 2021; Accepted 15 December 2021.

Immune Status of Osteoarthritis Patients

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Abstract

This study was performed for investigating of immune status among osteoartgritis patients through serological measurement of interleukin-1 (IL-1), IL-10, monocyte interferon gamma inducing factor (MIGF), p53 upregulated modulator of apoptosis (PUMA), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α) using the sandwich enzyme-linked immunosorbent assay (ELISA). Totally, 160 people involved 120 osteoarthritis patients and 40 healthy ones as a control, were subjected to sampling of venous blood. The total findings showed a significant elevation in values of IL-1 (147.5 ± 4.689 pg/ml), MIGF (241.5 ± 15 pg/ml), PUMA (57.70 ± 0.5358 pg/ml), and TNF-α (42.91 ± 0.839 pg/ml); whereas, there were significant decreases ($p<0.0338$) in values of IL-10 (102.6 ± 3 pg/ml) and TGF-β (91.56 ± 0.7646 pg/ml). Regarding age factor, patients of ≥36 years were reported a significant increase ($p<0.0086$) in values of PUMA (53.09 ± 0.8555 pg/ml) and TNF-α (44.89 ± 1.309 pg/ml), and a significant decrease ($p<0.0179$) in values of TGF-β (86.51 ± 0.9277 pg/ml) when compared to those of ≤35 years (49.72 ± 0.8222 pg/ml, 40.93 ± 0.9679 pg/ml, and 96.61 ± 0.4472 pg/ml, respectively). However, no significant variation ($p<0.4556$) was observed between both age groups (≤35 years and ≥36 years) relation to IL-1 (149.2 ± 6.188 pg/ml and 145.8 ± 7.116 pg/ml, respectively), IL-10 (102.9 ± 4.106 pg/ml and 102.3 ± 4.426 pg/ml, respectively) and MIGF (262.1 ± 18.31 pg/ml and 222.4 ± 22.26 pg/ml, respectively). Concerning gender, higher values of IL-10 was reported in females (112.9 ± 3.422 pg/ml) than males (92.25 ± 4.389 pg/ml), ($p<0.0002$); while, values of MIGF and PUMA were increased in males (270.2 ± 18.46 pg/ml, and 54.68 ± 0.7729 pg/ml, respectively) when compared to females (214.3 ± 21.09 pg/ml, and 48.13 ± 0.6383 pg/ml, respectively), ($p<0.0271$). However, insignificant variation ($p<0.9296$) in values of IL-1, TGF-β and TNF-α was appeared between males (143.9 ± 5.975 pg/ml, 91.70 ± 1.092 pg/ml, and 42.05 ± 1.178 pg/ml, respectively) and females (151.1 ± 7.260 pg/ml, 91.42 ± 1.084 pg/ml, and 43.78 ± 1.194 pg/ml, respectively). In conclusion, significant positive correlation between immune markers and osteoarthritis was confirmed in this study. However, furthermore studies are of great importance to support effect of other immune markers in initiation, degradation as well as healing of osteoarthritis.

Keywords: ELISA, MIGF, PUMA, TGF-β, TNF-α, Iraq

Introduction

Osteoarthritis is one of the older diseases, which affects mainly the elderly individuals causing synovial inflammation, bone deformation, loss and breakdown of articular cartilage;

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which collectively resulting in progressive joint failure, severe pain and experience loss of mobility (**1, 2**). Worldwide, osteoarthritis was detected in about 18% and 9.6% of women men, respectively who aged 60 years, and expected that approximately 76 million people living in the United States will have been diagnosed with the disease in the 2030s (**3**). As a result, work productivity is reduced along with rising costs to the health care system, and current available treatments prior to joint replacement were targeted to interarticular injections of hyaluronic acids and corticosteroids to ameliorate the symptoms (**4-6**). However, these therapeutic schedules doesn't showed to modulating an advancing of osteoarthritis because multi-factorial pathways that driven the set of components including cellular immunity responding to the inflammatory environments, biochemical cascades, and failing joint biomechanics (**7, 8**).

In general, immune system categorizes two parts; innate and adaptive, which are related to defenses of a body towards different risks like malignancies, injuries and microbes. Innate immunity is the first body responder which derived by macrophage and neutrophil; while in adaptive immunity, B- and T- cells in addition to antibodies comprise an acquired response (**9-11**). In chronic inflammation, macrophage and neutrophil revealed a complicated phenotype reflects divergenic activities among inflammatory and curing processes since activity of macrophages is detected throught osteoarthritis advancing whereas neutrophils having a predominant effect in pathogenesis of any disease (**12, 13**). Subsequently, several diseases could be resulted in impaired or incomplete polarization of macrophage which directed by a local microenvironment and cytokine signaling (**14**). Inflammatory cytokines have a significant effect in disease's advancing by stimulation a production of matrix metalloproteinase and increasing the degradation of this matrix (**15, 16**).

Imaging markers represent an important tool for evaluation of different diseases as well as for developing of drugs in a field. Nonetheless, widespread utilization of these diagnostic assays having limits due to lack a validated international score, availability and cost (**17**). Therefore, considerable attentions have given for identification promising biomarkers to detection of a disease and then treating of it by prospecting the risk role of each immune biomarker (**18**). Recent studies seek for understanding a driver and regulation of osteoarthritis, and naturally immune responsing of the body which influence osteoarthritis progression and then to heal it (**19-21**). Hence, this study aims to estimate immune status of osteoarthritic patients through serological measurement of IL-1, IL-10, MIGF, PUMA, TGF-β and TNF-α using the sandwich ELISA. Also, the association of immune markers among the individuals of study patient group to the most important risk factors; age and gender was studied.

Materials and methods

Ethical approval

The present study was licensed by, and carried out under the acceptance of the Scientific Committee of the Department of Biology, College of Science, University of Wasit (Wasit, Iraq).

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Samples collection

A total of 160 individuals; 120 osteoarthritis patients and 40 healthy as a control, were subjected to the current study. Of each one, 5 ml of venous blood was sampled under aseptic conditions into free-anticoagulant glass tube. Sera were collected after centrifugation of blood samples, and kept frozen until be tested. The study peoples were divided according to their age to two groups; ≤35 years and ≥36 years, and according to their genders to males and females.

Serology

Following the manufacturer instruction of the sandwich ELISA kits (Sunlong Biotech, China) of the targeted markers (IL-1, IL-10, MIGF, PUMA, TGF-β and TNF-α), the sera and the kit components of each marker were prepared, processed and the optical density (OD) was measured of 450 nm using the ELISA Reader (Bio Tek, USA). The concentration of each marker in serum samples was measured by plotting the concentrations and ODs of the Standard Solution of each marker in addition to ODs of the serum samples on the standard curve.

Statistical analysis

All obtained data were documented and analyzed statistically using the GraphPad Prism (*version* 6.0.1) Software. Statistically, the *t-test* was applied to analyzing the values of targeted markers of patient and healthy groups. Consequently, data regarding age and gender of study patients were estimated to detect their association with the values of targeted markers. Differences were considered significant at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***) and $p<0.0001$ (****), (**22**).

Results

Total results of immune markers

In this study, significant variation ($p<0.05$) was showed between values of patients and healthy groups (Figure 1). Significantly, increases in values of patient group were reported in IL-1 (147.5 ± 4.689 pg/ml), MIGF (241.5 ± 15 pg/ml), PUMA (57.70 ± 0.5358 pg/ml) and TNF-α (42.91 ± 0.839 pg/ml) when compared to values of healthy group (47.78 ± 1.529 pg/ml, 122.2 ± 4.846 pg/ml, 51.40 ± 0.6193 pg/ml, and 31.41 ± 0.6499 pg/ml, respectively); whereas, decreases ($p<0.0338$) in values of patient group were seen IN IL-10 (102.6 ± 3 pg/ml) and TGF-β (91.56 ± 0.7646 pg/ml) in comparison with those of healthy group (114.4 ± 3.816 pg/ml, and 99.25 ± 1.122 pg/ml, respectively).

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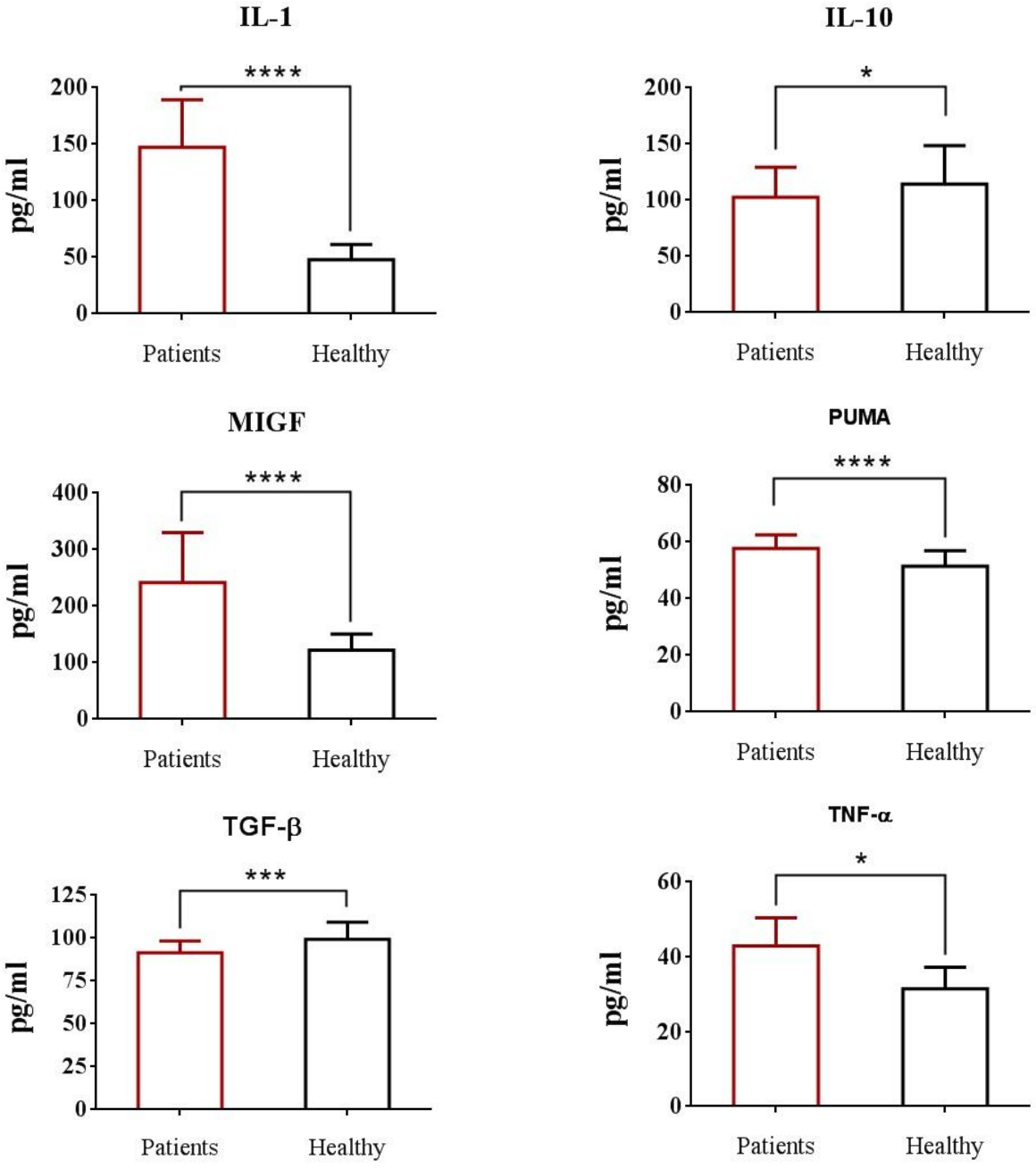

Figure (1): Total results for immune markers among individuals of patient and healthy groups

Association of immune markers to age of patient group

In patient individuals of ≥36 years, significant increases ($p<0.0086$) were reported in values of PUMA (53.09 ± 0.8555 pg/ml) and TNF-α (44.89 ± 1.309 pg/ml), while significant decreases ($p<0.0179$) were seen in values of TGF-β (86.51 ± 0.9277 pg/ml) when compared to those of ≤35 years (49.72 ± 0.8222 pg/ml, 40.93 ± 0.9679 pg/ml, and 96.61 ± 0.4472 pg/ml, respectively). However, no significant variation ($p<0.4556$) was observed between values of ≤35 years and ≥36 years regarding the IL-1 (149.2 ± 6.188 pg/ml and 145.8 ± 7.116 pg/ml, respectively), IL-10

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(102.9 ± 4.106 pg/ml and 102.3 ± 4.426 pg/ml, respectively) and MIGF (262.1 ± 18.31 pg/ml and 222.4 ± 22.26 pg/ml, respectively), (Figure 2).

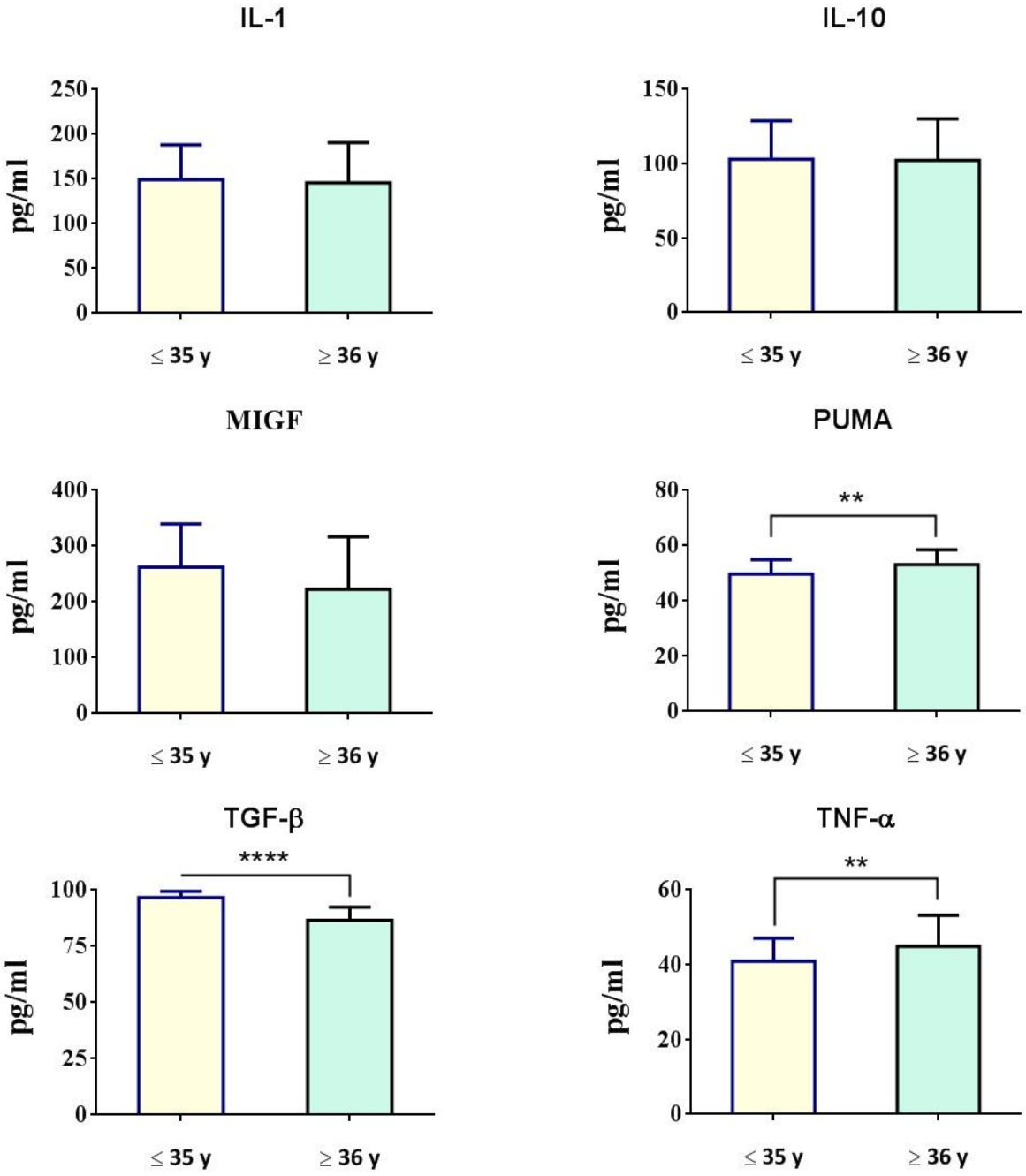

Figure (2): Association of immune markers to age of patient group

Association of immune markers to gender of patient group

Significantly, higher values of IL-10 was reported in females (112.9 ± 3.422 pg/ml) than males (92.25 ± 4.389 pg/ml), ($p<0.0002$); while, values of MIGF and PUMA were increased in

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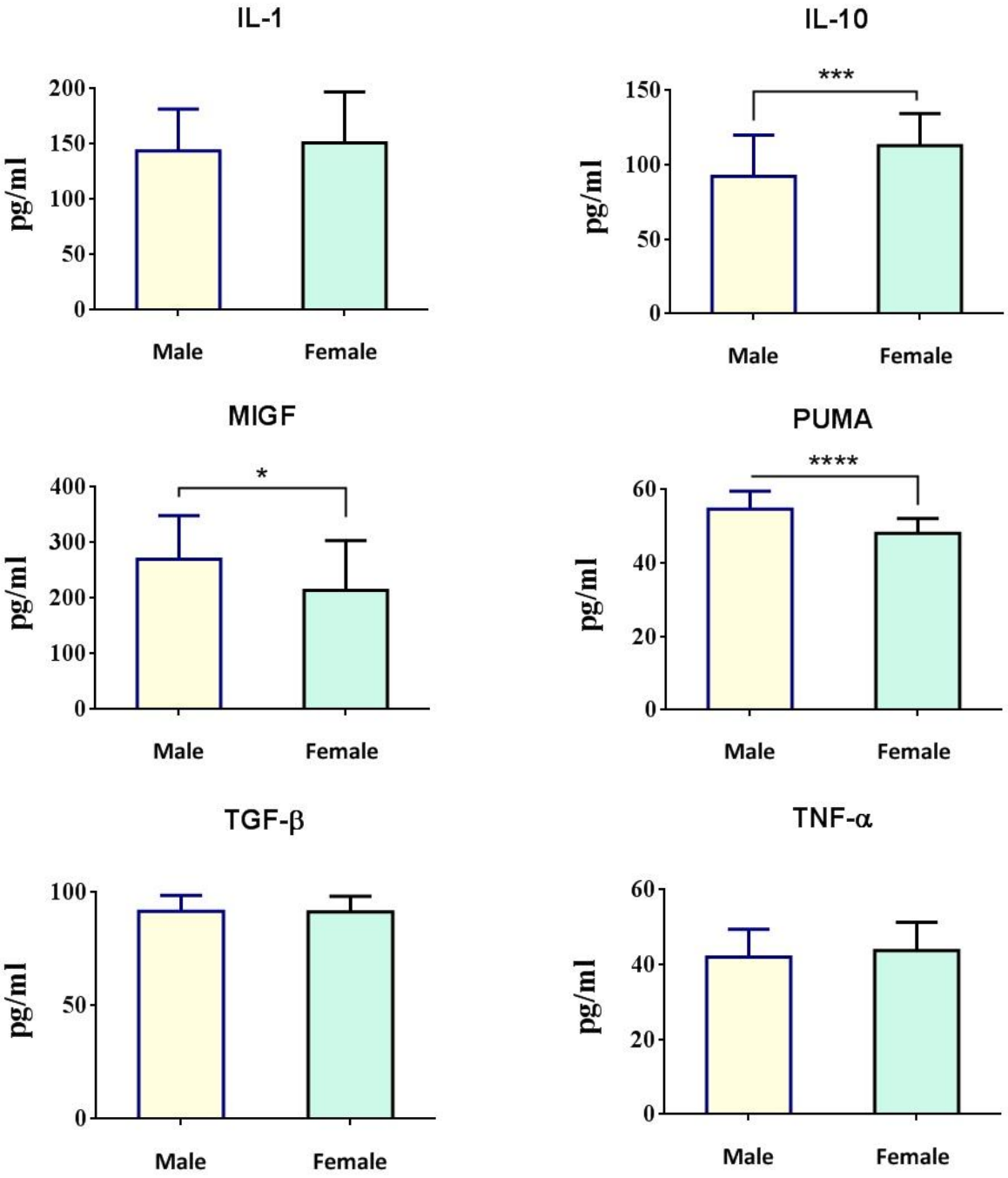

Figure (3): Association of immune markers to gender of patient group

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Discussion

In almost cases, osteoarthritis affects the structures of a whole joint resulting in a progressively many changes in bones, ligaments, and cartilages in addition to synovial inflammation. Therefore, immune markers have been modified for quantification of a disease progression and joint remodeling (**17**). In this study, serological investigation of some immune markers revealed that the values of these markers were differed significantly since significant increase were recorded in levels of IL-1, MIGF, PUMA, and TNF-α; while significant reduces were observed in levels of IL-10 and TGF-β. In two studies, the authors have mentioned to the roles of inflammatory and pro-inflammatory cytokines in particular IL-1 and TNF-α, in expressing and responding of synovial cells and chondrocytes to the disease, and their implication in pathological progressive of osteoarthritis (**23, 24**). Syx et al. (2018) demonstrated the role of inflammatory cytokines in osteoarthritic individuals may be directly participated in generation of pain their actions on innervations of joint nociceptors (**25**). Chondrocytes were showed to be synthesized different components and upregulation of IL-1 and TNF-α which both known to induce cartilage breakdown (**26, 27**). Guo et al. (2015) revealed that generating biologically active IL-1 can induce pyrooptosis or inflammatory cell death (**28**). Multiple crystals including calcium phosphate, calcium pyrophosphate and uric acid were found to activating inflammasomes causing a releases of IL-1 into synovial fluid targeting synovial lining cells and chondrocytes with suppression of extracellular matrix synthesis and upregulation of cartilage degrading enzymes and finally destruction of a joint (**29, 30**). TNF-α has observed to influencing and coordinating the inflammatory responses in almost tissues through activation of signaling cascades, with influencing the excitability of nociceptors either by expression of downstream cytokines or direct affecting on tissues (**31, 32**). In addition, TNF-α has the ability to producing long-term alterations by expression of several molecules in sensory nerves which act as a link in pain behavior signaling pathway (**33**). Monocytes consider the main source for deriving of many cells and cytokines, which having different activities in blood circulation and tissues. In addition to TNF-α, interferon-gamma (IFN-γ) is one of the most important cytokines which affected osteoarthritis cartilage by the upregulation of IL-1 (**34**). Garcia et al. (2021) documented the positive correlation between osteoarthritis and the concentrations of IL-1, TNF-α and IFN-γ suggesting their roles in synovial inflammation and osteophyte formation (**35**).

Significant role of PUMA in upregulation in osteoarthritis cartilage has been confirmed by several studies (**21, 36, 37**). Structural analysis had shown that PUMA exerts pro-apoptotic activity by binding to Bcl-2 family members and by mediating their mitochondrial localization, which release the mitochondrial apoptogenic proteins leading to caspase activation and cell death (**38-40**). In a previous study, Li et al. (2012) indicated that PUMA induction may be an important mechanism leading to chondrocyte apoptosis and may contribute to the resulting osteoarthritis suggesting the role of PUMA as a potential target for anti-apoptotic treatments in osteoarthritis (**41**).

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Cartilage damage is a major problem in osteoarthritis, and growth factors like TGF-β are highly pleiotropic cytokines having great potential in cartilage repair, wound healing, angiogenesis and immunoregulation (**42**). In different studies, the findings confirmed that the TGF-β was expressed in high levels in normal cartilage, but was almost absent in osteoarthritis cartilage (**43-45**). While, other researchers have been found that the blocking of TGF-β made cartilage more susceptible to damage (**46**), and lack of TGF-β causes a reduction in extracellular matrix deposition, and suppression of catabolic stimuli is drastically reduced (**47, 48**). In both rheumatoid arthritis and osteoarthritis, several researchers have been shown the increased levels of T regulatory cells that play a significant role in lowering the secretion of IL-10, likely due to decreasing of mucin domain-containing-3 and decreasing an expression of T cell inhibitory receptor and T cell immunoglobulin (**49-51**).

It is well accepted that aging is an important contributing factor to the development of osteoarthritis, and the mechanism responsible appear to be multi-factorial and may include an age-related pro-inflammatory state that has been termed "inflamm-aging" (**52**). In this study, levels of PUMA and TNF-α were correlated significantly with increasing age; while, TGF-β was reduced with advanced age. Many studies have been referred to that systemic inflammation is promoted by aging changes in adipose tissue resulting in an increased production of cytokines in particular TNF-α (**53-55**). In older adults with osteoarthritis, a number of studies showed a significant increasing in levels of TNF-α (**56**), and high levels of soluble receptors for TNF-α correlated with decreased physical ability of osteoarthritis patients (**52**). Xiong et al. (2015) supported the link between age-related cell apoptosis and PUMA signaling, suggesting that this factor might serve as a potential target for age-related diseases treatment (**57**). Cheema et al. (2015) documented the apoptosis and necrosis mediate skeletal muscle fiber loss in age-induced mitochondrial enzymatic abnormalities (**58**). Radak et al. (2017) reported that changes in cellular homeostasis may trigger either necrosis or apoptosis which often depends on cell type, cell age and location in the body (**59**).

In females, our findings reported that level of IL-10 was increased significantly; while, values of MIGF and PUMA were decreased significantly suggesting that negative impact of osteoarthritis in males is more than observed in females. These findings were in agreement with the results by other studies (**60-62**), but in contrast with those reported by another (**63, 64**). However, variation in immune markers between males and females might be attributed to sex hormones that determine immune response, lifestyle variables, and stress factor.

Conclusion

Significant positive correlation between immune markers and osteoarthritis was confirmed in this study. Also, our study indicated that PUMA may be a new diagnostic and therapeutic target for osteoarthritis. Based on our data, it appeared that immune markers are age-related, and negative impact of osteoarthritis is more prominence in males than females. However,

furthermore studies are necessary to investigate the role of other immune markers in initiation, degradation and healing of osteoarthritis.

Competing Interests

No competing interests.

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Received 08 November 2021; Accepted 15 December 2021.

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