ISSN: 1607-8322, e-ISSN: 2220-5799 Anaesthesia, Pain & Intensive Care
Vol 28(4); August 2024 DOI: 10.35975/apic.v28i4.2501

ORIGINAL RESEARCH PAIN MANAGEMENT

Evaluation of vascular endothelial growth factor and interleukin-1 receptor antagonists of synovial fluid cytokines in patients with anterior cruciate ligament injury, meniscal tear and cartilage damage

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ABSTRACT

Background & objective: Knee pain, related to osteoarthritis (OA) of the knees is a disease of middle to old age. The males are the predominant victims. It has been suggested that knee diseases may be related to inflammatory biomarkers such as vascular endothelial growth factor (VEGF-A) and interleukin-1 (IL-1Ra) receptor antagonists of synovial fluis cytokines. These biomarkers might provide diagnostic and prognostic information to the clinicians. We investigated the levels of VEGF-A and IL-1Ra in patients known to suffer from suffered from anterior cruciate ligament (ACL) injuries, meniscal tears, and cartilage damage.

Materials and methods: A total of 76 Iraqi participants were diagnosed to be suffering from anterior cruciate ligament (ACL) injuries, meniscal tears, and/or cartilage damage during the period from November 2022 to May 2023. They were admitted to the Al-Furat Al-Awsat Private Hospital in the Al-Qadisiyah Governorate, Iraq. The patients were divided into three groups according to the duration of the disease: Group I included patients with injuries for less than 6 months, Group II with 6-12 months and Group III had patients with injuries for more than one year. The enzyme-linked immunosorbent assay (ELISA) technique was used to detect inflammatory markers VEGF-A and and IL-1Ra. Data were summarized, and Microsoft Office Excel 2013 and GraphPad Prism 9.2.0 were used to present the statistical analysis.

Results: Patients in Group I showed a significantly elevated level of VEGF-A (372.2 ± 68.95 pg/mL) as compared to Group II (273.5 ± 53.47 pg/mL) and Group III (232.2 ± 28.22 pg/mL) ($P < 0.0001$). While the concentration of IL-1Ra declined in patients with Group III (162.1 ± 7.036 pg/mL), as compared with both Group II (173.1 ± 9.351 pg/mL) and Group I (200 ± 34.53 pg/mL) with significant differences ($P < 0.0001$).

Conclusion: The current study identified two synovial fluid biomarkers, vascular endothelial growth factor (VEGF-A) and interleukin-1 (IL-1), receptor antagonists of synovial fluis cytokines, whose concentrations after anterior cruciate ligament injury differ depending on the duration of the injury.

Abbreviations: ACL - anterior cruciate ligament; OA **-** osteoarthritis; IL-1Ra - interleukin-1 receptor antagonists; VEGF-A - vascular endothelial growth factor

Keywords: Vascular Endothelial Growth Factor; interleukin-1 Receptor Antagonists; Anterior Cruciate Ligament

Citation: Abd Ali AJ, Alzaniily AA, AL-Ardi I. Evaluation of vascular endothelial growth factor and interleukin-1 receptor antagonists of synovial fluid cytokines in patients with anterior cruciate ligament injury, meniscal tear and cartilage damage. Anaesth. pain intensive care 2024;28(4):616–619; **DOI:** 10.35975/apic.v28i4.2501

Received: December 16, 2023; **Reviewed:** April 13, 2024; **Accepted:** April 24, 2024

1. INTRODUCTION

The anterior cruciate ligament (ACL) is an important ligament that supports the knee joint. The tibia and femur are joined by the ACL. It is 7-12 mm wide and 32 mm long.[1] The ACL is made up of the posterolateral bundle (PLB) and the anteromedial bundle (AMB). The primary difference between them is that the AMB lengthens while the PLB shortens during flexion, proving that they are not isometric.[2]

The ACL is made up of a microstructure made up of several types of collagen bundles (mostly type I) and a matrix made up of a network of proteins, glycoproteins, elastic systems, and glycosaminoglycans with complicated functional interactions. The ACL is innervated by posterior articular tibia nerve branches and nourished by the middle radicular artery branches.[3]

Vascular endothelial growth factor (VEGF), formerly known as vascular permeability factor, is the primary regulator of both pathological and healthy angiogenesis.[4] The most researched VEGF isoform, VEGF-A, is involved in endothelial proliferation, migration, and survival.[5] Endogenous VEGF-A supports endothelial homeostasis, which is the preservation of the balance of intercellular and intracellular factors/parameters in order for the endothelium to function properly. VEGF-A has a high level of activity with vascular endothelial cells, particularly through interactions with the VEGF-R1 and -R2 receptors present on the endothelial cell membrane. However, it has also an impact on a variety of different cell types, including monocyte/macrophage migration, neurons, cancer cells, and kidney epithelial cells.[6]

Angiogenesis is a necessary phase in the healing process that aids in the repair and remodeling of wounded tissue, but can have harmful consequences if unregulated. Many cytokines and growth factors, including VEGF, influence angiogenesis.[7]

The interleukin-1 receptor antagonist (IL-1Ra) is an IL-1 family member that binds to IL-1 receptors but causes no intracellular response. In arthritis, colitis, and granulomatous pulmonary illness, IL-1Ra is an essential natural anti-inflammatory protein.[8] In the presence of pre-existing OA, IL-1Ra significantly and safely reduces pro-inflammatory cytokine concentrations while retaining cartilage integrity, indicating a feasible therapeutic option for postponing traumatic osteoarthritis (PTOA) in young patients with ligament or meniscal damage.[9]

2. METHODOLOGY

This cross-sectional study was performed from November 2022 to May 2023, after approval by the Health and Medical Human Research Ethics Committee, University of Al-Qadisiyah, Iraq (No. 4409/30).

A total of 76 participtant were enrolled in Al-Qadisiyah Governorate's at AL-Furat AL-Awsat Pravite Hospital, and written informed consents were ontained from them.

Exclusion criteria included; any other ligament injury requiring surgical treatment, cartilaginous and osteochondral lesions, bones fractures, degenerative meniscus tears, previous knee surgery, previous meniscus injury**, c**hronic inflammatory diseases within the joint or outside the joint, immune diseases or tumors**.**

Diagnosis was made by a senior orthopaedic surgeon, based on the current history, physical examination, magnetic resonance imaging (MRI), and confirmation by arthroscopic examination. The history of patients included the age and body mass index. A total of 76 Iraqi participants suffered from ACL injuries, meniscal tears, and cartilage damage; and divided into three groups according to the duration of the disease: less than six months for Group I (25 patients), 6-12 months for Group II (26 patients), and more than one year for Group III (25 patients). During the ACL restoration process, synovial fluid was taken from each patient's injured knee.

Human VEGF-A and IL-1Ra-1F3 were detected by ELISA (Elabscience, USA).

2.1. Statistical analysis

The data was compiled using GraphPad Prism 9.2.0 and Microsoft Office Excel 2013 for the statistical analysis. The data were shown as the means ± standard deviations in numerical form. To find significant group differences, post hoc analysis with the Tukeys test and one-way ANOVA were employed. $P < 0.05$ indicated that all of the data were considered significant.

3. RESULTS

3.1. VEGF-A levels

The preesent study showed increased levels of VEGF-A (372.2 ± 68.95 pg/mL) in patients with ACL injury in Group III as compared to Group I (273.5 ± 53.47 pg/mL), but less than Group II (232.2 ± 28.22 pg/mL).

Significant difference was indicated in mean values between Group I and Group II ($P = 0.0233$); significant difference in mean values between Group I and Group III ($P < 0.0001$) and a significant difference in mean values between Group II and Group III ($P < 0.0001$) as shown in Figure 1.

3.2. IL-1Ra levels

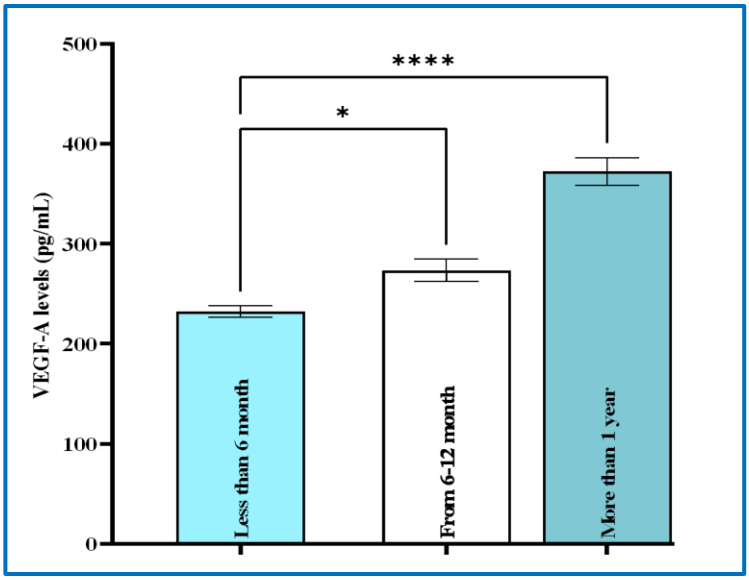

Figure 1: VEGF-A levels (pg/mL) in the groups. A substantial difference (P < 0.0001) was observed. * Significant differences compared to all groups, P ≤ 0.05, data shown as Means ± SD.

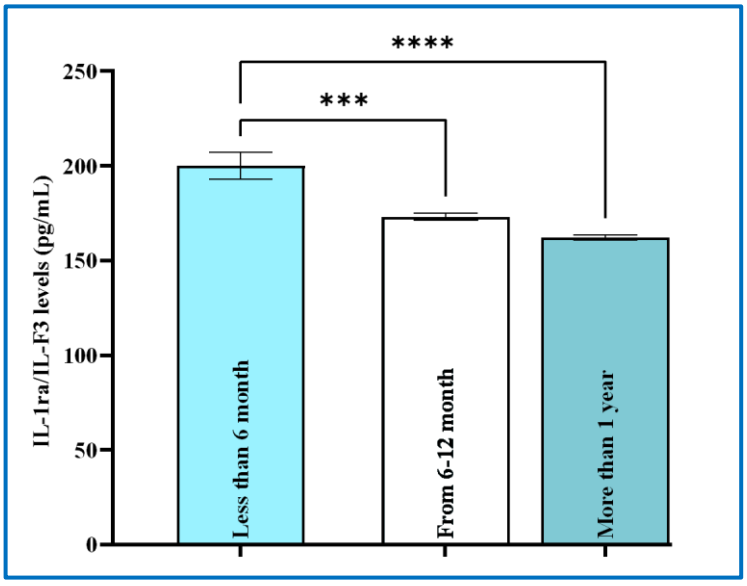

Figure 2: Estimation of concentrations of IL-1ra/IL-F3 levels (pg/mL) A comparison between group. A substantial difference (P 0.0001) was found when comparing all groups. Data are given as means standard deviations. shows *significant differences relative to all groups, P 0.05.

The results of this study showed a decline in levels of IL-1Ra/IL-F3 (162.1 ± 7.036 pg/mL) in patients with ACL injury for more than 1 year (Group III), compared with Group II (173.1 ± 9.351 pg/mL) and Group I (200 ± 34.53 pg/mL). The differences were significant (P < 0.0001).

Significant difference was present in mean values between Group I and Group II (P = 0.0001) and Group III (P < 0.0001) and there was a non-significant difference in mean values between Group II and Group III (P = 0.1743) As shown in Figure 2.

The comparison of the levels of VEGF and IL-1Ra among all groups are shown in Table 1. VEGF-A levels were very low in patients Group II compared with other groups. A significant difference was observed (P < 0.0001). IL-1Ra/IL-F3 levels were very high in patients Group II compared with other groups; differences were significant (P < 0.0001). Comparison of mean values of VEGF-1 and IL-1Ra with Tukey's multiple comparison test is presented in Table 2.

4. DISCUSSION

VEGF-A is an angiogenic factor that is released by chondrocytes, synoviocytes, and endothelial cells.[10] It has been demonstrated to be expressed in the articular cartilage of joints afflicted by ACL injuries.[11] The present study has showed a significant elevation in the VEGF-A levels in patients with an injury period more than one year after ACL injury as compared with patients with an injury period less than one year. Increased levels of the angiogenesis-induceing growth factor VEGF-A may be associated with the Pro-inflammatory cytokines and more severe cartilage damage.[12]

Our findings in this study are mostly similar with recent research on VEGF-1 levels in ACL injuries. According to certain researches, VEGF-A overexpression may elevate matrix metalloproteinase (MMP) expression levels, thus jeopardizing extracellular matrix (ECM) equilibrium.[13] Previous research have demonstrated that a positive correlation between measuring synovial fluid concentration for VEGF-A and the duration of ACL injury.[14]

Similarly, in a study has been desecribe by Walsh and his colleaques, that proved the duration of ACL injury could increase rate of VEGF-A.[15] It is believed that VEGF-A exerts its effects by binding to VEGFR1 and VEGFR2, the levels of which are elevated in neuropathic pain and hyperalgesia. But the effect of the duration is still unclear, necessitating more study with a bigger sample size.[16]

Table 1: Comparison of mean values of the studied biomarkers among all groups

Biomarkers	Group I (n = 24)	Group II (n = 25)	Group III (n = 24)	P value
VEGF-A level (pg/ml)				
Range	202.4-298.4	205.4-399.4	234.4-496.4	< 0.0001****
Mean ± SD	232.2 ± 28.22	273.5 ± 53.47	372.2 ± 68.95	
IL-1Ra/IL-F3 level (pg/ml)				
Range	163.7-305.7	159.7-199.7	138.7	< 0.0001****
Mean ± SD	200 ± 34.53	173.1 ± 9.351	162.1 ± 7.036	

*****Highly significant*

Table 2: Comparison of mean values of VEGF-1 and IL-1Ra with Tukey's multiple comparison test

Groups	Summary	Adjusted P value
VEGF-A level (pg/ml)		
< 6 months vs 6-12 months	*	0.0233
< 6 months vs > 1 y	****	< 0.0001
6-12 months vs > 1 y	****	< 0.0001
IL-1Ra/IL-F3 level (pg/ml)		
< 6 months vs from 6-12 months	***	< 0.0001
< 6 months vs > 1 y	****	< 0.0001
6–12-months vs > 1 y	ns	0.1743

** Significant; ***Very significant; ****Highly significant; ns - Not significant*

Few researches have concentrated on ACL rips, which differ from chronic cases in the way they displayed an early inflammatory response.[17]

In agreement with our study, Seatan and his colleaques have been reported that the level of VEGF-A in synovial fluid of patients with ACL is tenfold increased. Also, Glabella and his group, they have studied 20 patients with primary ACL, proved a statistically significant increase in the level of synovial VEGF-A.[18] Establishing a comparable biomarker profile for ACL injuries may be crucial because of the variability of the injury mechanism and consequent intra-articular inflammatory environment.[19]

When a knee is injured, IL-1Ra is released into the synovial fluid, causing an acute inflammatory response that usually lasts 24 to 48 hours but can sometimes last for several weeks.[20] In our current study, our participants were classified into three groups of patients with anterior cruciate ligament injuries at different periods, and it was found that the level of IL-1Ra decreased as the injury period increased. These low levels of the anti-inflammatory cytokine IL-1ra imply a suppressed anti-inflammatory response. An overexpression of pro-inflammatory cytokines in ACL injury could be associated with a low expression of IL-1ra that could drive biochemical degeneration of articular cartilage.[21] Our results are consistent with a study showing that within 1 week following ACL injury, patients had a greater synovial fluid concentration of IL-1Ra.[22]

Also, the ACL patients showed reduced levels of IL-1Ra in their synovial fluid over the course of two months to over a year. After one year of damage, the concentration of IL-1Ra in synovial fluid was consistently reduced.[23] Early rises in the anti-inflammatory IL-1ra may be a sign that the body is trying to balance the pro-inflammatory response that was brought on by the joint injury. Nevertheless, IL-1ra concentrations declined with increasing time from injury, which may suggest that the body is less able to shield bone, cartilage, and synovial tissue from degradation agents over an extended period of time.[24] However, increases in IL-1Ra are due to key inflammatory cytokines involved in ACL injury.[25]

5. CONCLUSION

The current study identified two synovial fluid biomarkers, vascular endothelial growth factor (VEGF-A) and interleukin-1 (IL-1), receptor antagonists of synovial fluis cytokines, whose concentrations can indicate the duration of the injury.

6. Data availability

The numerical data generated during this research is available with the authors.

7. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

8. Authors' contribution

All authors took part in the concept, conduct of the study and manuscript preparation.

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