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RESEARCH ARTICLE

NLRP3 Expression in Peripheral Blood Mononuclear Cells of Patients with Rheumatoid Arthritis

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SUMMARY

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by painful, swollen, and inflamed joints. Individual, genetic, and environmental factors influence the development of the disease, which causes involvement not only in the joints but also in other extra-articular tissues. However, the etiopathogenesis of the disease has not yet been fully elucidated. NLRP3, a crucial component of the innate immune system, may contribute to recurrent and chronic inflammation, resulting in inflammation-related diseases. Therefore, we aimed to examine the expression pattern of NLRP3 in peripheral blood mononuclear cells isolated from patients with RA using immunocytochemical approaches in this study. Our findings demonstrated that NLRP3 expression was significantly increased in patients with RA compared to the healthy controls ($p<0.01$). The highest expression among the patient groups was observed in the active period cases, and this expression considerably increased compared to the control group ($p<0.01$). Patients in the remission period possessed the lowest expression among the patient groups. Since female gender is considered an independent risk factor for the disease, the effect of gender on expressions was also investigated. NLRP3 was expressed at higher levels in female patients than in males; however, this difference was not significant ($p>0.05$). The expression patterns of the patient and control groups suggest that NLRP3 may be involved in the development and progression of the disease. To our knowledge, our study is the first research investigating NLRP3 expression profile in peripheral blood mononuclear cells obtained from patients with RA using immunocytochemical approaches. The results of our study highlight significant aspects. However, further research using more sensitive methods with a larger number of cases is required to assess the function of NLRP3 in RA and to provide a deeper insight into the mechanism.

Key Words: *Inflammasome, inflammation, NLRP3, rheumatoid arthritis.*

Romatoid Artrit Hastalarının Periferik Kan Mononükleer Hücrelerinde NLRP3 Ekspresyonu

ÖZ

Romatoid artrit (RA), esas olarak ağrılı, şiş ve iltihaplı eklemlerle karakterize sistemik bir otoimmün hastalıktır. Sadece eklemlerde değil diğer eklem dışı dokularda da tutuluma neden olan hastalığın gelişiminde bireysel, genetik ve çevresel faktörler etkilidir. Ancak hastalığın etiyopatogenezi henüz tam olarak aydınlatılamamıştır. Doğuştan gelen bağışıklık sisteminin önemli bir bileşeni olan NLRP3, tekrarlayan ve kronik iltihaplanmaya katkıda bulunarak iltihaplanma ile ilişkili hastalıklara neden olabilir. Bu nedenle, bu çalışmada RA'lı hastalardan izole edilen periferik kan mononükleer hücrelerinde NLRP3'ün ekspresyon profilini immünositokimyasal yaklaşımlar kullanarak araştırmayı amaçladık. Bulgularımız RA'lı hastalarda NLRP3 ekspresyonunun sağlıklı kontrollere göre anlamlı düzeyde yüksek olduğunu gösterdi ($p<0.01$). Hasta grupları arasında en yüksek ekspresyon hastalığın aktif döneminde olan vakalarda bulundu ve bu ekspresyon kontrol grubuna göre oldukça artmıştı ($p<0.01$). Remisyon dönemindeki hastalar, hasta grupları arasında en düşük ekspresyona sahipti. Kadın cinsiyet hastalık için bağımsız bir risk faktörü olarak düşünüldüğünden, cinsiyetin ekspresyon üzerindeki etkisi de araştırıldı. NLRP3 kadın hastalarda erkeklerden daha yüksek seviyelerde eksprese oldu; ancak bu fark anlamlı değildi ($p>0.05$). Hasta ve kontrol gruplarının ekspresyon paternleri, NLRP3'ün hastalığın başlangıcında ve ilerlemesinde rol oynayabileceğini düşündürmektedir. Bildiğimiz kadarıyla çalışmamız, RA'lı hastalardan elde edilen periferik kan mononükleer hücrelerde immünositokimyasal yaklaşımlar kullanılarak NLRP3 ekspresyon profilinin araştırıldığı ilk araştırmadır. Çalışmamızın sonuçları önemli hususları vurgulamaktadır. Ancak, RA'da NLRP3'ün işlevini açıklamak ve mekanizmaya daha derin bir bakış açısı sağlamak için daha fazla sayıda vakayla daha hassas yöntemler kullanarak yürütülecek yeni araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: *İnflamazom, inflamasyon, NLRP3, romatoid artrit.*

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by pain, swelling, inflammation, redness, and joint deformities. RA, one of the most prevalent autoimmune diseases in the world, primarily affects the inner surface of the synovial joints (Debreova et al., 2022). Joint destruction starts in the first stages of the disease, progresses rapidly, and causes irreversible damage, functional insufficiency, and progressive disability. Some patients also develop extra-articular manifestations, involvement, and inflammation in non-articular tissues. Thus, patients are at increased risk of inflammatory, ocular, pulmonary, nervous, renal, dermal, cardiovascular disease, and even cancer (Smolen et al., 2018; Piantoni & Ohrndorf, 2023). The onset and progression of the disease, triggered by many individual, genetic, and environmental factors, increases the abnormal immune response. Gender, immunological disorders, gut microbiota, traumas, infectious factors, smoking, hormonal changes, stress, eating habits, and even socioeconomic factors play roles in the etiology of the disease (Deane et al., 2017; Arleevskaya et al., 2022).

T-cells, B-cells, and macrophages are inflammatory cells involved in the pathology of RA. T-cells activate macrophages and fibroblasts, causing them to become tissue-destroying cells. These cells exacerbate joint inflammation by boosting cytokines and chemokines (Yap et al., 2018). Inflammatory mediator release and the interaction of cells in the joint synovium lead to the destruction of synovial tissues. Increased inflammation also causes the activation of endothelial cells and the expression of leukocyte adhesion molecules. T-cells, B-cells, and monocytes spread to the peripheral blood after invading the synovial membrane and other areas of the joints (Aletaha & Smolen, 2018; Manning, Lewis, Marsh & McGettrick, 2021; Ding et al., 2023).

There are three clinical phases in RA in which immunoregulation impairs. Autoantibody levels rise, and autoimmunity develops without visible clinical symptoms in the initial phase. An autoimmune response in inflammatory cells augments in the second phase, characterized by inflammatory attacks on the joints and other clinical signs of RA. In the third phase, local inflammatory responses increase, and permanent destruction, deformation, and chronic inflammation are observed (Holmdahl, Malmström & Burkhardt, 2014).

Inflammasomes are sizeable multimolecular protein structures located in the cytosol of immune cells. An inflammasome complex consists of three proteins: a receptor, an adapter, and an effector (Barnett, Li, Liang & Ting, 2023). Nod like receptor protein 3 (NLRP3) inflammasome, a vital part of the innate immune system, is a multi-protein complex activating many inflammatory responses. It is an assembly made of NLRP3 as a sensor protein, Apoptosis-associated speck-like protein (ASC) as an adapter protein, and caspase-1 as an effector protein. Both endogenous and exogenous sources activate NLRP3 inflammasome. This activation can result in the release of higher levels of pro-inflammatory cytokines and other inflammatory factors, which contribute to inflammatory diseases (Wang et al., 2020; Zheng, Liwinski & Elinav, 2020; Chen et al., 2023; Leu et al., 2023).

Early diagnosis of the disease and initiation of treatment is very crucial. Treatment becomes increasingly challenging, and joint damage becomes irreversible as the disease progresses to the chronic phase. Various biomolecular pathways have been investigated and proposed, but the etiology and pathophysiology of RA are not fully understood. Moreover, there is no specific biomarker to diagnose the disease. Therefore, in the present study, our goal was to examine the impact of NLRP3 in patients with RA who had varying disease activity using immunocytochemical approaches.

MATERIAL AND METHODS

Study group

Our study comprised fifty-six patients diagnosed with RA by the rheumatologist. Thirty individuals without autoimmune diseases, including RA, were in the control group. The study group was selected among people who applied to the Rheumatology Clinic of the Ministry of Health Ankara Atatürk Training and Research Hospital. Male and female participants aged 18-65 years, approved by the rheumatologist, were included in the study. The patient group was classified into three subgroups based on the period of the disease and the medication. The first group was the remission period patients (n=11). The second group was the active period patients (n=16). These two groups of patients had started medications for RA when they were included in the study. The third group included newly diagnosed patients who had not yet started drug treatment for the disease (n=29). Clinical Research Ethics Committee of Ankara University approved the study with the number 13-605-16 (25.07.2016). The study was conducted under the Declaration of Helsinki and informed consent was obtained from the participants.

Peripheral mononuclear cells (PBMCs) isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood samples. Whole blood samples were diluted with phosphate-buffered saline (PBS) in the same volume and layered onto Ficoll-Paque media. Then, the samples were centrifuged at 2300 rpm for twenty min, and the phase, including PBMCs, were taken and washed with PBS. The centrifuge was applied at 1600 rpm for ten min, and the bottom cells were resuspended.

Immunocytochemistry

Isolated PBMCs were prepared for the immunocytochemistry analysis, and the following steps were performed (Oğuztüzün et al., 2011; Şimşek et al., 2012). The samples were put on slides coated with poly-L-lysine. Prepared slides were subjected to cytocentrifugation at 1000 rpm for five min. Preparations removed from the centrifuge were fixed in alcohol. Distilled water was applied to the slides and washed for one-two min. Then, the slides were exposed to H_2O_2 for ten min. The slides were washed with distilled water for two-three min followed by washing with PBS for five min (three times). A protein-blocking solution was applied to the slides for ten min. Afterward, the slides were incubated for one hour with the primary antibody solution. Different dilution ratios of primary antibody solution were applied to obtain the best result. The antibody ratio of 1:200 was applied. PBS was applied to the slides for washing three times for five min. Secondary antibodies were administered for ten min. Then, the slides were washed three times for five min with PBS. The streptavidin-HRP complex was applied to the slides for ten min. PBS was used for washing three times for five min. The slides were exposed to DAB solution for ten min. The slides were washed with distilled water for two-three min and then kept in hematoxylin for a short time. Then the slides were dehydrated in graded concentrations of alcohol (45, 50, 60, 70, 80, 90, 96, and 100%) and were kept in xylol after this process. Finally, they were covered with Entellan® and left to dry. After drying, the slides were made ready for examination. Immunocytochemical evaluations were performed in light microscopy (Olympus Corporation U-DO3). The expression levels were determined as (0) for negative staining (no protein expression) and (+1) for positive staining (with protein expression). The slides were photographed with an Olympus Corporation Digital camera (C-7070).

Statistical analysis

IBM SPSS Statistics V.25.0 program (IBM Corp., Rel. 2017, Armonk, NY) was used for data analysis and statistical evaluations. Categorical data were expressed as the numbers (percentage). Continuous data were presented as the mean ± standard error of the mean (SEM). The data distribution was evaluated with the Shapiro-Wilk test. The Levene test was used

to test the homogeneity of the variances. The Mann-Whitney U test was carried out to compare the data of two groups, and the Kruskal-Wallis test was used for more than two groups. The Chi-square test was performed to compare categorical data, and Fisher's exact test was used when more than 20% of cells had an expected frequency of five or less. Spearman's rank correlation test was carried out for the correlation analysis. A p-value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Study group

Our study group, which included eighty-six subjects in total, consisted of fifty-six patients with RA as the patient group and thirty healthy cases as the control group. The patient group consisted of three subgroups. The first group included patients with RA in the remission (naive) period (remission group) (n=11). The second group included patients with RA in the active period (active group) (n=16). The third group included non-treated newly diagnosed patients with RA (newly diagnosed group) (n=29).

The demographic and clinic data of the patients are explained in Table 1. The mean age of the patient group was 43.71 years, while the control group had a mean age of 38.17 years. There were forty-three females and thirteen males in our patient group and nineteen females and eleven males in the control group. The smoking habits of the participants were recorded with questionnaires. They were classified into three groups: those who had never smoked, those who had quit smoking, and those who were active smokers. 16.07% of the patient group and 33.33% of the control group stated that they had never smoked. The number of people who quit smoking was five in both groups. 75% of the patients and 50% of the control group reported as active smokers. The medication usage and other diseases of the participants were recorded with questionnaires. While 93.33% of the control group did not use any medications, 50% of the patient group did. The most commonly used medication combination in the patient group was methotrexate (MTX), hydroxychloroquine, and steroids, with sixteen cases. 90% of the control group did not suffer from any disease, and 91.07% of the patient group had only RA.

Immunocytochemistry

NLRP3 protein expression levels were evaluated by immunocytochemical analysis in the PBMCs of the patient and control groups. Positive staining was detected in 45% of the patient group, while no positive staining was observed in the control group. The NLRP3 protein expressions of the patient group were significantly higher than that of the control ($p<0.01$). Moreover, NLRP3 expression patterns were evaluated according to disease activity in the patient group. The highest expression was observed in patients with active RA. An average of 50% positive staining was detected in the active-period patients, and that was significantly higher when compared to the control group ($p<0.01$). The newly diagnosed group displayed almost 46% positive staining. This expression was noticeably increased compared to the control group ($p<0.01$). The lowest expression among the patient group, with a mean positive staining of 33%, belonged to the patients in remission. All three patient groups showed higher expressions than the control group; however, no significant difference within the patient group was noted ($p>0.05$).

Immunocytochemical NLRP3 expression levels of the control and patient groups are shown in Figure 1. Immunocytochemical scores were also determined according to the disease activity in the patient group.

Table 1. Demographic and clinic characteristics of the cases in patient and control group

Data		Patient group (n=56)	Control group (n=30)	*P-value*	*X^2 value*
Age		43.71±10.22 (24-63)	38.17±13.40 (20-62)	*>0.05*[a]	-
Gender					
Female		43 (76.79%)	19 (63.33%)	*>0.05*[b]	*1.757*
Male		13 (23.21%)	11 (36.67%)		
Smoking habits					
Never smokers		9 (16.07%)	10 (33.33%)	*>0.05*[b]	5.483
Former smokers		5 (8.93%)	5 (16.67%)		
Active smokers		42 (75.00%)	15 (50.00%)		
Medication					
No		28 (50.00%)	28 (93.33%)	*<0.001**[c]	23.857
Yes		28 (50.00%)	2 (6.67%)		
	Abatasept	1 (1.79%)	0		
	Adalimumab and lymphlunomide	1 (1.79%)	0		
	Etanercept and leflunomide	2 (3.57%)	0		
	MTX, hydroxychloroquine, and steroid	16 (28.57%)	0		
	MTX and rituximab	3 (5.36%)	0		
	NSAID	2 (3.57%)	0		
	Tofacitinib	2 (3.57%)	0		
	Tocilizumab	1 (1.79%)	0		
	Vitamin	0	2 (6.67%)		
Other diseases					
No		51 (91.07%)	27 (90.00%)	>0.05[c]	2.087
Yes		5 (8.93%)	3 (10.00%)		
	HT	3 (5.36%)	1 (3.33%)		
	HT and HL	1 (1.79%)	0		
	DM	1 (1.79%)	2 (6.67%)		

The categorical data was expressed as a number (percentage). The continuous data was presented as mean ± standard deviation (SD). DM: diabetes mellitus, HT: hypertension, HL: hyperlipidemia, MTX: methotrexate, NSAID: non-steroidal anti-inflammatory drug. *p<0.05, [a]Mann-Whitney U test, [b]Chi-square test, [c]Fisher's exact test.

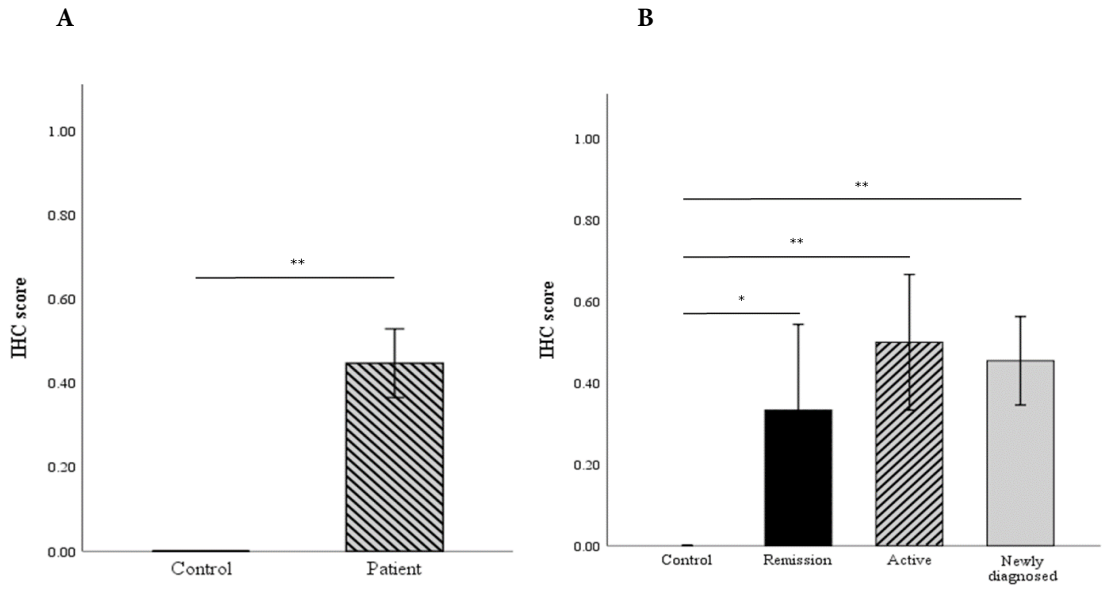

Figure 1. Immunocytochemical NLRP3 expression profile of control and patient groups

The bars represent mean ± SEM (95 %CI), Chart A: The patient group was compared to the control using the Mann-Whitney U test. Chart B: Remission, active, and newly diagnosed patients were compared to the control using the Kruskal-Wallis test. *p<0.05, **p<0.01.

Light microscopy images of NLRP3 positive and negative staining are presented in Figure 2.

NLRP3 expression profiles of the patients based on their gender, age, and smoking habits were evaluated, and the results are shown in Figure 3. Higher expression was observed in female patients than in male cases, as seen in Figure 3A. The mean score was 0.48±0.09 in female patients and 0.38±0.18 in male ones. However, the difference between the groups concerning gender was not statistically significant (p>0.05).

Patients were grouped as younger than 30 years old, 30-50 years old, and older than 50 years old. The effect of age on expression levels was examined (Figure 3B). There was no significant difference in NLRP3 expression levels based on patients' age. Correlation analysis was also carried out to determine the relationship between their age and the expressions. No statistically significant correlation was found between age and expression levels when examined without grouping as control or patient, with a correlation coefficient of 0.124 (p>0.05).

NLRP3 expression profile was evaluated according to patients' smoking habits, and the results are illustrated in Figure 3C. Patients who had never smoked showed the lowest expression, with a score of 0.29±0.18. The mean score was 0.60±0.24 and 0.46±0.10 for former and active smokers in the patient group, respectively. However, these differences were not statistically significant (p>0.05).

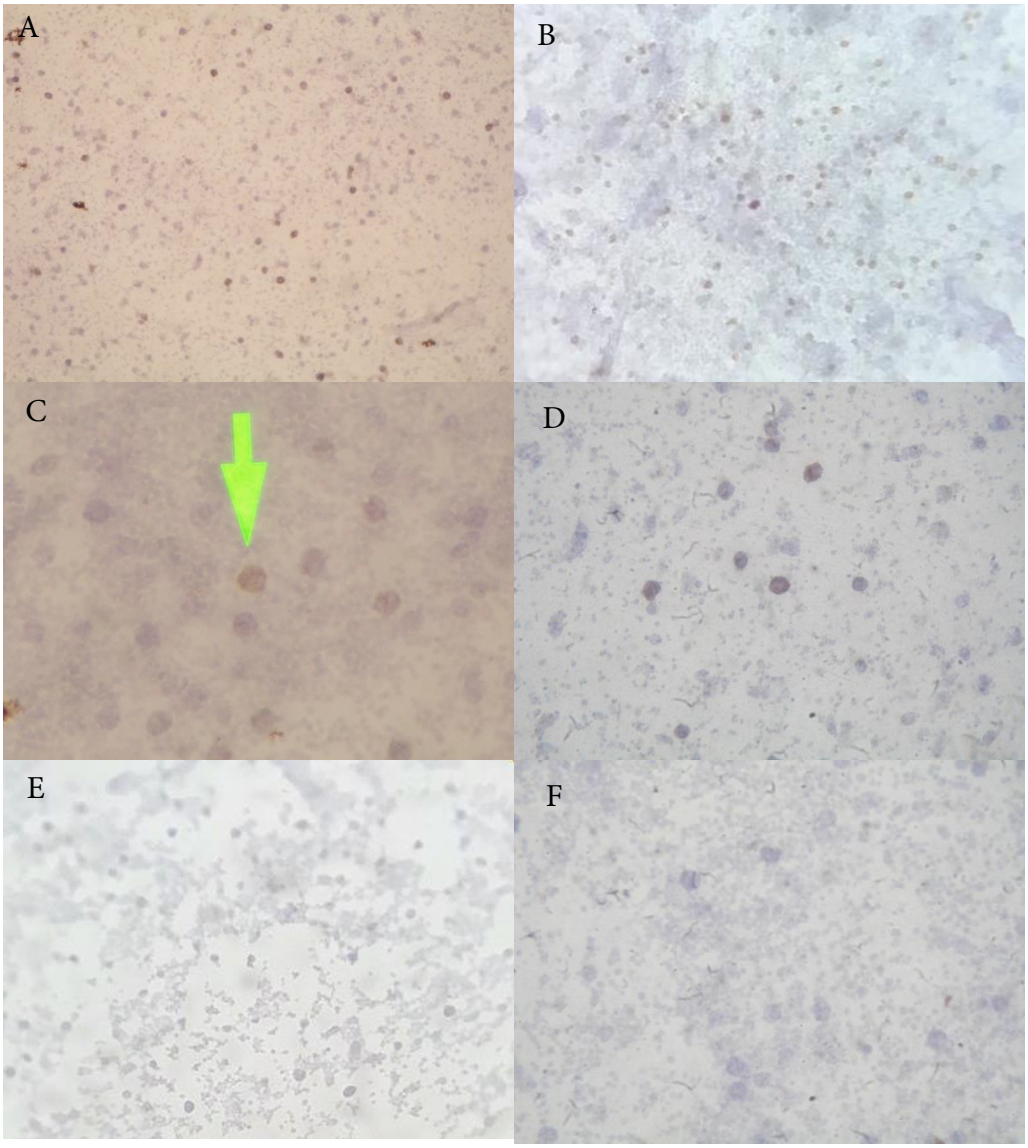

Figure 2. Light microscopy images of NLRP3 protein staining in PBMCs of the patient group

A: Positive staining (magnification x40), B: Positive staining (magnification x40), C: Positive staining (magnification x100), D: Positive staining (magnification x100), E: Negative staining (magnification x40), F: Negative staining (magnification x100).

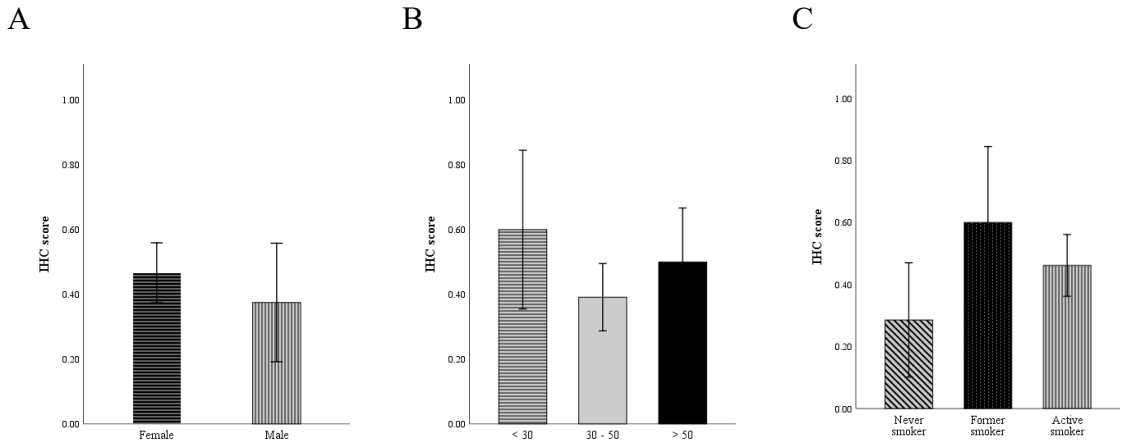

Figure 3. NLRP3 expression of the patients with RA based on their gender, age, and smoking habits

The bars represent mean ± SEM (95 %CI). Comparisons were made by gender, age, and smoking habits, respectively. Chart A: Gender (Mann-Whitney U test). Chart B: Age (Kruskal-Wallis test). Chart C: Smoking habits (Kruskal-Wallis test).

Female gender, smoking, occupational dust exposure, air pollution, low vitamin D levels, dysregulation of gut microbiota, periodontal diseases, and obesity are important risk factors for RA. The disease activity and course are more severe in females. Puberty, pregnancy, lactation, menopause, and factors such as hormone replacement therapy and oral contraceptive use can also affect hormone levels and disease activity (Smolen et al., 2018; Yu et al., 2020). Therefore, the effect of gender and smoking habits was also examined in our study. Female patients possessed higher expression of NLRP3 than males. The expression level of active and former smokers was higher than those who never smoked. However, these gender- and smoking-related differences were not statistically significant, which may be due to the number of cases. New studies are needed to examine the other factors that may contribute to the formation and development of the disease.

NLRP3 inflammasome consists of NLRP3 protein, ASC protein, and pro-caspase-1, which mediates the initial innate immune response to cellular injury and stress. NLRP3 inflammasome induces the conversion of pro-inflammatory cytokines pro-IL-1β and pro-IL-18 to their active forms and subsequently triggers the release of caspase-1 (Zhang, Yang, Li & Zhao, 2021). This inflammasome can be activated by endogen sources such as cholesterol crystals within macrophages in atherosclerosis, free fatty acids and lipids in adipose tissue in obesity and diabetes, β-amyloids in Alzheimer's disease, and also glucose, ATP, calcium influx, mitochondrial dysfunction, and monosodium urate (Jin & Fu, 2019; Fusco, Siracusa, Genovese, Cuzzocrea & Di Paola, 2020; Zhan, Li, Xu, Xiao & Bai, 2023). Chemical irritants, crystal particles, ultraviolet rays, bacteria, and viruses can also activate it as exogen sources. This process induces oxidative stress and inflammation, resulting in inflammation-related diseases (Abderrazak et al., 2015; Antushevich, 2020). RA, mainly characterized by painful and swollen joints, is one of the most crucial inflammation-related diseases in terms of its incidence and progression profile (Sparks, 2019). Environmental and genetic factors contribute to the onset and development of RA; however, the etiopathogenesis of the disease remains unclear. Similar to RA, NLRP3 can also be activated by several environmental factors (Fusco et al., 2020). Therefore, it is considered that the factors that trigger NLRP3 could contribute to RA. Moreover, no biomarker allows a definitive diagnosis of the disease.

The progression of RA can be classified into two periods: active and remission. Long-term untreated active disease causes irreversible joint destruction and extra-articular manifestations. Thus, one of the goals of the treatment is to reduce the severity of the disease and ensure the transition of patients from active to remission form (Aletaha et al., 2005; Ajeganova & Huizinga, 2017). From this point of view, we aimed to examine the expression profile of NLRP3 on PBMCs isolated from patients with RA in the current study. The effect of disease activity on this expression was also determined. Our results displayed that NLRP3 protein expression of the patient group was significantly higher than that of the control group. Another evaluation was made by classifying the patient group regarding disease activity and medications. The highest expression was observed in treated patients with active RA followed by newly diagnosed patients who had not started RA medication yet. The lowest expression was found in patients in remission. Our results are supported by several articles that have focused on the function and mechanism of NLRP3 in RA. Choulaki et al. (2015) demonstrated that NLRP3 inflammasome activity, basal intracellular levels of NLRP3, and NLRP3-mediated active caspase-1, pro-IL-1β, and active IL-1β expressions were increased in peripheral blood cells of patients with active RA compared to the control group. NLRP3 inflammasome has been shown to activate CD4 T-cells of RA patients and regulate Th17 cell differentiation, resulting in adaptive immunological dysfunction (Zhao, Gu, Zeng & Wang, 2018). IL-6 overactivated NLRP3 by creating synergy with pentaxin 3, the crucial part of innate immunity, and its ligand, complement C1q, in patients with RA (Wu et al., 2020). DNA polymerase-β expression significantly decreased in PBMCs of active RA patients and collagen-induced arthritis mice, while NLRP3, IL-1β, and IL-18 expressions upregulated in mice. These levels increased the incidence of RA, DNA damage, macrophage infiltration, and bone destruction (Gu et al., 2022). Gene expression and genetic polymorphism studies have also been carried out to determine the role of NLRP3 in RA. Some NLRP3-related single-nucleotide polymorphisms and gene polymorphisms have been reported to influence RA susceptibility and response to TNF inhibitors (Mathews et al., 2014; Li et al., 2023).

CONCLUSION

NLRP3, a vital component of the innate immune system, may contribute to recurrent, and chronic inflammation, resulting in inflammation-related diseases. Our results showed that NLRP3 was expressed higher in patients with RA compared to healthy cases and may be effective in the onset and pathogenesis of the disease. High expression in patients with active RA suggests that NLRP3 could also contribute to the progression of the disease. All these data point to significant findings; however, new studies with more cases and more sensitive methods are needed to confirm our results and provide further insight into the mechanism.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Concept and Design: S.Y.S, O.K., C.N., L.D.K., S.O., T.Ç.; Supervision: T.Ç.; Resource: S.Y.S, O.K., C.N., L.D.K., S.O., T.Ç.; Materials: S.Y.S, O.K., C.N., L.D.K., T.Ç.; Data Collection/Processing: S.Y.S, O.K., C.N., L.D.K., G.G.Ş., S.O., T.Ç.; Analysis/ Interpretation: S.Y.S, O.K., C.N. , L.D.K., P.K., G.G.Ş., S.O., T.Ç.; Literature Search: S.Y.S., T.Ç.; Writing: S.Y.S., T.Ç.; Critical Reviews: T.Ç.

REFERENCES

Abderrazak, A., Syrovets, T., Couchie, D., El Hadri, K., Friguet, B., Simmet, T. & Rouis, M. (2015). NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. *Redox Biology, 4,* 296-307. doi:10.1016/j.redox.2015.01.008

Ajeganova, S. & Huizinga, T. (2017). Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. *Therapeutic Advances in Musculoskeletal Disease, 9*(10), 249-262. doi:10.1177/1759720X17720366

Aletaha, D., Ward, M. M., Machold, K. P., Nell, V. P., Stamm, T. & Smolen, J. S. (2005). Remission and active disease in rheumatoid arthritis: defining criteria for disease activity states. *Arthritis & Rheumatism, 52*(9), 2625-2636. doi:10.1002/art.21235

Aletaha, D. & Smolen, J. S. (2018). Diagnosis and management of rheumatoid arthritis: a review. *Jama, 320*(13), 1360-1372. doi:10.1001/jama.2018.13103

Antushevich, H. (2020). Interplays between inflammasomes and viruses, bacteria (pathogenic and probiotic), yeasts and parasites. *Immunology Letters, 228*, 1-14. doi:10.1016/j.imlet.2020.09.004

Arleevskaya, M., Takha, E., Petrov, S., Kazarian, G., Renaudineau, Y., Brooks, W., ... Novikov, A. (2022). Interplay of environmental, individual and genetic factors in rheumatoid arthritis provocation. *International Journal of Molecular Sciences, 23*(15), 8140. doi:10.3390/ijms2315814

Barnett, K. C., Li, S., Liang, K. & Ting, J. P. Y. (2023). A 360° view of the inflammasome: Mechanisms of activation, cell death, and diseases. *Cell, 186*(11), 2288-2312. doi:10.1016/j.cell.2023.04.025

Chen, Y., Ye, X., Escames, G., Lei, W., Zhang, X., Li, M., … Yang, Y. (2023). The NLRP3 inflammasome: contributions to inflammation-related diseases. *Cellular & Molecular Biology Letters, 28*(1), 51. doi:10.1186/s11658-023-00462-9

Choulaki, C., Papadaki, G., Repa, A., Kampouraki, E., Kambas, K., Ritis, K., … Sidiropoulos, P. (2015). Enhanced activity of NLRP3 inflammasome in peripheral blood cells of patients with active rheumatoid arthritis. *Arthritis Research & Therapy, 17*(1), 257. doi:10.1186/s13075-015-0775-2

Debreova, M., Culenova, M., Smolinska, V., Nicodemou, A., Csobonyeiova, M. & Danisovic, L. (2022). Rheumatoid arthritis: from synovium biology to cell-based therapy. *Cytotherapy, 24*(4), 365-375. doi:10.1016/j.jcyt.2021.10.003

Deane, K. D., Demoruelle, M. K., Kelmenson, L. B., Kuhn, K. A., Norris, J. M. & Holers, V. M. (2017). Genetic and environmental risk factors for rheumatoid arthritis. *Best Practice & Research Clinical Rheumatology, 31*(1), 3-18. doi:10.1016/j.berh.2017.08.003

Ding, Q., Hu, W., Wang, R., Yang, Q., Zhu, M., Li, M., ... Zhu, Y. Z. (2023). Signaling pathways in rheumatoid arthritis: implications for targeted therapy. *Signal Transduction and Targeted Therapy, 8*(1), 68. doi:10.1038/s41392-023-01331-9

Fusco, R., Siracusa, R., Genovese, T., Cuzzocrea, S. & Di Paola, R. (2020). Focus on the role of NLRP3 inflammasome in diseases. *International Journal of Molecular Sciences, 21*(12), 4223. doi:10.3390/ijms21124223

Gu, L., Sun, Y., Wu, T., Tang, X, Zhao, L., He, L., ... Guo, Z. (2022). A novel mechanism for macrophage pyroptosis in rheumatoid arthritis induced by Pol β deficiency. *Cell Death & Disease*, *13*(7), 583. doi:10.1038/s41419-022-05047-6

Holmdahl, R., Malmström, V. & Burkhardt, H. (2014). Autoimmune priming, tissue attack and chronic inflammation—the three stages of rheumatoid arthritis. *European Journal of Immunology, 44*(6), 1593-1599. doi:10.1002/eji.201444486

Jin, Y. & Fu, J. (2019). Novel insights into the NLRP 3 inflammasome in atherosclerosis. *Journal of the American Heart Association, 8*(12), e012219. doi:10.1161/JAHA.119.012219

Leu, S. Y., Tsang, Y. L., Ho, L. C., Yang, C. C., Shao, A. N., Chang, C. Y., ... Tsai, Y. S. (2023). NLRP3 inflammasome activation, metabolic danger signals, and protein binding partners. *Journal of Endocrinology, 257*(2), e220184. doi:10.1530/JOE-22-0184

Li, R. N., Ou, T. T., Lin, C.H., Lin, Y.Z., Fang, T.J., Chen, Y. J... & Yen, J.H. (2023). NLRP3 gene polymorphisms in rheumatoid arthritis and primary Sjogren's syndrome patients. *Diagnostics (Basel), 13*(2), 206. doi:10.3390/diagnostics13020206

Manning, J. E., Lewis, J. W., Marsh, L. J. & McGettrick, H. M. (2021). Insights into leukocyte trafficking in inflammatory arthritis–imaging the joint. *Frontiers in Cell and Developmental Biology, 9,* 635102. doi:10.3389/fcell.2021.635102

Mathews, R. J., Robinson, J. I., Battellino, M., Wong, C., Taylor, J. C., Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS), … McDermot, M. F. (2014). Evidence of NLRP3-inflammasome activation in rheumatoid arthritis (RA); genetic variants within the NLRP3-inflammasome complex in relation to susceptibility to RA and response to anti-TNF treatment. *Annals of the Rheumatic Diseases*, *73*(6), 1202-10. doi:10.1136/annrheumdis-2013-203276

Oğuztüzün, S., Sezgin, Y., Yazıcı, S., Fırat, P., Özhavzalı, M. & Özen, H. (2011). Expression of glutathione-S-transferases isoenzymes and p53 in exfoliated human bladder cancer cells. *Urologic Oncology: Seminars and Original Investigations*, *29*(5), 538-44. doi:10.1016/j.urolonc.2009.08.001

Piantoni, S. & Ohrndorf, S. (2023). Rheumatoid arthritis: pathogenesis and target-treatments. *Frontiers in Medicine, 10,* 1145163. doi:10.3389/fmed.2023.1145163

Smolen, J. S., Aletaha, D., Barton, A., Burmester, G. R., Emery, P., Firestein, G. S., ... Yamamoto, K. (2018). Rheumatoid arthritis. *Nature Reviews Disease Primers, 4,* 18001. doi:10.1038/nrdp.2018.1

Sparks, J. A. (2019). Rheumatoid arthritis. *Annals of Internal Medicine, 170*(1), ITC1-ITC16. doi:10.7326/AITC201901010

Şimşek, G., Oğuztüzün, S., Güreşçi, S., Kılıç, M., Bozkurt, Ö. F. & Ünsal, A. (2012). The expression of GST isoenzymes in acinar adenocarcinoma, intraepithelial neoplasia, and benign prostate tissue: correlation of clinical parameters with GST isoenzymes. *Turkish Journal of Biology, 36,* 687-693. doi:10.3906/biy-1203-31

Wang, Z., Zhang, S., Xiao, Y., Zhang, W., Wu, S., Qin, T., ... Li., L. (2020). NLRP3 inflammasome and inflammatory diseases. *Oxidative Medicine and Cellular Longevity, 2020*, 4063562. doi:10.1155/2020/4063562

Wu, X., Li, K., Yang, H., Yang, B., Lu, X., Zhao, L., ... Zhang, X. (2020). Complement C1q synergizes with PTX3 in promoting NLRP3 inflammasome over-activation and pyroptosis in rheumatoid arthritis. *Journal of Autoimmunity, 106,* 102336. doi:10.1016/j.jaut.2019.102336

Yap, H. Y., Tee, S. Z. Y., Wong, M. M. T., Chow, S. K., Peh, S. C. & Teow, S. Y. (2018). Pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. *Cells*, *7*(10), 161. doi:10.3390/cells7100161

Yu, C., Liu, C., Jiang, J., Li, H., Chen, J., Chen, T. & Zhan, X. (2020). Gender differences in rheumatoid arthritis: interleukin-4 plays an important role. *Journal of Immunology Research, 2020,* 4121524. doi:10.1155/2020/4121524

Zhan, X., Li, Q., Xu, G., Xiao, X. & Bai, Z. (2023). The mechanism of NLRP3 inflammasome activation and its pharmacological inhibitors. *Frontiers in Immunology, 13,* 1109938. doi:10.3389/fimmu.2022.1109938

Zhang, Y., Yang, W., Li, W. & Zhao, Y. (2021). NLRP3 inflammasome: checkpoint connecting innate and adaptive immunity in autoimmune diseases. *Frontiers in Immunology, 12,* 732933. doi:10.3389/fimmu.2021.732933

Zhao, C., Gu, Y., Zeng, X. & Wang, J. (2018). NLRP3 inflammasome regulates Th17 differentiation in rheumatoid arthritis. *Clinical Immunology, 197*, 154–60. doi:10.1016/j.clim.2018.09.007

Zheng, D., Liwinski, T. & Elinav, E. (2020). Inflammasome activation and regulation: toward a better understanding of complex mechanisms. *Cell Discovery, 6*(1), 36. doi:10.1038/s41421-020-0167-x