The Role of TNF- α in Pathogenesis of Rheumatoid Arthritis

Hadeel Abbas Abed
Biotechnology Department, College of Science, University of Baghdad ,Baghdad, Iraq, had_alkhafaji@outlook.com

Asmaa Mohammed Saud
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Tumor necrosis factor alpha (TNF-α) is one of the essential cytokines that have an important role in the pathophysiology of rheumatoid arthritis (RA); it is a chronic inflammatory disease characterized by a disordered immune system that predominantly affects multiple peripheral joints. The study aimed to evaluate the level of serum TNF-α as a biomarker of pathogenesis in RA. In the Medicine City Baghdad hospital consulting clinic, blood samples were taken from 150 volunteers: 119 rheumatoid arthritis patients, 22 (18,.3%) males and 97 (80.8%) females and 31 healthy controls, 12 (38.7%) males and 19 (61.3%) females. The Demographic characteristics showed statistically significant results for sex, age, family history, smoking, duration of disease, and DAS-28 between rheumatoid arthritis patients and healthy control, where the P-value (>0.001) and BMI (0.038). Further, the results showed significance in TNF- α in the patients' group compared to the control group. In addition, TNF-α relates to demographic characteristics in age, BMI, and biological therapy with p-value (>0.001, 0.038, >0.001), respectively. According to the area under the curve for RF (0.76) and anti-CCP (0.61) was not associated with disease activity score-28.

Keywords: *Rheumatoid arthritis, TNF- α, ELISA, Cytokines.*

INTRODUCTION

Rheumatoid arthritis is a common autoimmune disease (Shrivastava et al., 2015). That is associated with progressive disability, systemic complications, and possibly early death. The immune system in rheumatoid arthritis, the synovium is seen as a foreign organism instead of seeing it as a self-tissue (Burska et al., 2014). Studies reveal an evaluation of inflammatory cytokines in rheumatoid arthritis (Zhang, 2021) and to understand the balance between stimulatory and inhibitory mechanisms in inflammatory conditions such as TNF-α, IL-1, and IL-6 have an essential role in the pathogenesis of rheumatoid arthritis (Pala et al., 2018). Many proinflammatory cytokines such as TNF-α, IL-1, and IL-6 are in all patients (Inam Illahi et al., 2021). It is discouraged to some degree by the manufacture of cytokine inhibitors such as IL-1ra and soluble TNF-R and anti-inflammatory cytokines such as IL-10 and TGF-β. But this is not sufficient as unable to counteract all the cytokines produced (Isomaki and Punnonen, 1997). Other proinflammatory cytokines were inhibited, which neutralized TNF-α, leading to the concept that the proinflammatory cytokines are linked with TNF-α, the dominant central regulator of IL-1(McGonagle et al., 2018). This theory has been successfully experienced in animal models. These studies have provided the basis for clinical trials to find suitable anti-TNFα therapy in patients with chronic rheumatoid arthritis. Anti-tumor necrosis factor placebo antibodies were used and showed remarkable medical benefit,

confirming the theory that TNFα is important in rheumatoid arthritis (Huang et al., 2019). Retreatment research has also shown benefits in recurrent relapses, indicating that the disease remains TNFα dependent (Farrugia and Baron, 2016). These studies demonstrate that cytokine expression and regulation analysis may yield effective therapeutic targets in inflammatory diseases. The cytokine level in patients with RA indicates illness. It may be the way to treat this disease through the use of drugs that work by injecting antibodies against tumor necrosis factor-alpha (Arend and Dayer, 1995). This indicates the primary role of this cytokine in the pathogenesis of chronic arthritis, but the main cause is not yet known (Klareskog et al., 2020). The aim of this study was to assess the levels of serum TNF-α as a potential biomarker of prognosis in RA and evaluate the correlation of serum TNF-α with demographic characteristics.

Materials and Methods

Subjects

The study was conducted from March 2022 to May 2022 after approval from the Ethical Review Committee of Baghdad University (Refcode: CSEC/0222/0023). one hundred fifty volunteers from the case-control study were divided into two groups: the first group had 119 blood samples which were taken from Rheumatoid arthritis patients (22 males and 97 females) with an age range are 21-77 years while the other group has 31 blood samples taken from healthy individuals (12 males and 19 females) and age range 22-73 years. Blood samples were taken from the medicine city Baghdad hospital teaching, the consulting clinic, unit of rheumatology in Baghdad, Iraq. Patients were diagnosed based on the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA (Aletaha et al., 2010). All participants gave informed consent and completed a questionnaire about each patient and control information. A certified, trained rheumatologist did clinical examinations. Exclusion criteria for healthy controls and patients included individuals with other immune diseases.

TNF-α serum concentration measurement

A three ml venous blood sample was taken from each patient in a gel tube and separated in centrifuged at 3000 rpm to get the serum. The serum level of TNF-α was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (MBS2502004; My BioSource, San Diego, USA).

Statistical Analysis

Data analysis was done on Statistical Package for Social Sciences (SPSS) windows, version 26 (IBM SPSS Statistics, Armonk, NY). The normality test Shapiro–Wilk was used to determine whether the studied parameters followed a Gaussian distribution. Data appeared as median ± standard deviation (SD). Mann-Whitney was used to compare the median of the studied groups. Regression Analysis to an estimation of relationships between variables. In addition, ROC was used to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC), were as well calculated. In all statistical analyses ($p < 0.05$) was measured statistically significant.

Results

Demographic characteristics in study groups

Blood samples were taken from 150 patients: including rheumatoid arthritis patients 119,

representing 79.33% of samples, including males 2(18.3 %) and females 97 (80.8%), with Mean± S.D (54.33±12.872) years. While the remaining 31 represent 20.6% of samples were taken from healthy people as a control sample 12(38.7%) males and 19(61.3%), and the mean± S.D age range, was (41.94±12.450) years. The demographic characteristics included sex, age, family history, smoking, duration of disease, and DAS-28 with (P>0.001) and BMI (0.038). In addition, females showed a higher significance of rheumatoid arthritis, while the age range (21-77) has more affected by rheumatoid arthritis with a Mean± S.D age of (54.33±12.872) years. The demographic and clinical characteristics for patients and healthy controls are summarized in (Table1).

Table 1: Demographic and clinical characteristics of patient's Rheumatoid arthritis and healthy control.

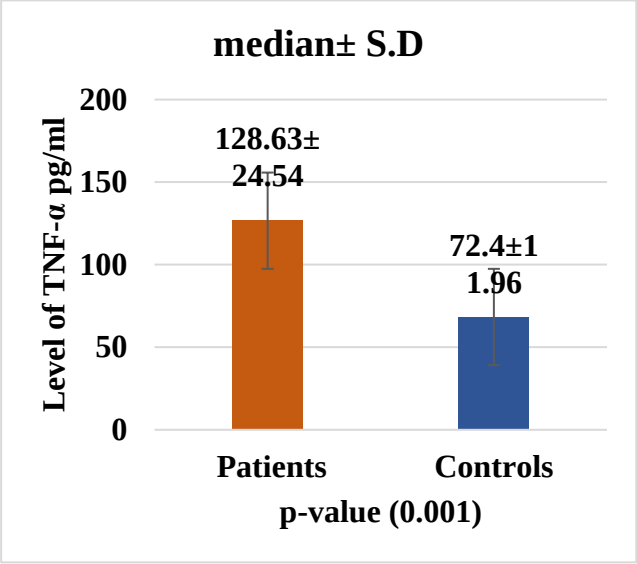
Demographic characteristics		Patient RA		Healthy Control		P- value
		No.	Percent%	No.	Percent%	
Age	**20 - 40**	15	12.6%	17	54.8%	>0.001
	41 - 61	60	50.4%	13	41.9%	
	>77	44	37%	1	3.2%	
	Mean± S.D	54.33±12.872		41.94±12.450		
Sex	**Female**	97	80.8%	19	61.3%	>0.001
	Male	22	18.3%	12	38.7%	
Family history	**No**	97	80.8%	31	33.7%	>0.001
	Yes	22	18.3%	---	--------	
Smoker	**No**	103	103%	22	71.0%	>0.001
	Yes	16	16%	9	29.0%	
BMI	**Normal 18.5-24.9**	18	15.1%	7	21.6%	0.038
	Overweight 25-29.9	45	37.8%	14	45.2%	
	Obese 30-34.5	44	36.9%	9	29%	
	Extremely Obese 35<	12	10.1%	1	3.2%	
Duration of Disease	**<10**	79	66.4%	---	---------	>0.001
	10-19	24	20.2%	---	---------	
	20-30	16	13.4%	---	---------	

DAS-28	**Low**	3	2.5%	---	---------	>0.001
	Medium	63	52.9%	---	---------	
	High	53	44.5%	---	---------	
Total		119		31		

Level serum of TNF-α in study groups

The median serum levels of TNF-α significantly increase among patients RA compared to healthy groups, where the p-value (0.001), is shown in (Figure1).

Figure 1: Level TNF–α in patients and control groups.



When comparing TNF-α and demographic characteristics between patient RA and healthy control show significance each for age, and BMI, and there was also a significant difference between patient groups that take biological medication and those that do not, as illustrated in (Table 2).

Table 2: The comparison of serum levels of TNF-α and study variables.

Demographic characteristics		**TNF- α in Patient RA**		**TNF- α in healthy Control**		**p-value**
		NO.	**Median, (min-max).**	**NO.**	**Median, (min-max).**	
Sex	**Female**	97	123.3, (84.9 -231.6)	19	72.9, (58.9 -91.2)	0.75
	Male	22	142.7, (113.8 -167.6)	12	63.3, (60.0 -95.7)	
age	**20 - 40**	15	64.5, (58.9 -95.7)	17	66.4, (60.0 -95.7)	>0.001

	41 - 61	60	121.2, (92.3 -167.6)	13	68.6, (58.9 -90.60)	
	>77	44	132.1, (84.9 -166.6)	1	82.2, (82.2 -82.2)	
Family history	**No**	97	127.3, (84.9 -231.6)	31	68.3, (58.9 -95.7)	0.213
	Yes	22	117.4, (92.3 -154.2)	---	--------	
Smoker	**No**	103	124.9, (84.9 -231.6)	22	72.8, (58.9 -91.2)	0.601
	Yes	16	146.8, (92.5 -164.2)	9	66.0, (61.0 -95.7)	
BMI	**Normal**	18	134.4, (92.5 -167-6)	7	72.7, (61.0 -91.2)	0.038
	Overweight	45	128.1, (96.5 -166.5)	14	68.3, (58.9 -95.7)	
	Obese	44	117.4, (84.9 -157.8)	9	72.8, (60.0 -94.9)	
	Extremely Obese	12	124.1, (92.3 -231.6)	1	61.9, (61.9 -61.9)	
Duration of Disease	**>10**	24	127.6, (92.5 -231.6)	---	----------	0.611
	10 - 20	12	124.2, (84.9 -166.5)	---	----------	
	> 20	10	122.1, (105.6 -154.3)	---	----------	
DAS-28	**Low**	3	100.3, (118.1 -132.9)	---	----------	0.348
	Moderate	75	128.1, (98.1 -231.6)	---	----------	
	High	41	132.8, (84.9 -166.6)	---	----------	
Biologics Therapy	**No**	112	135.1, (112.9 -167.6)	31	68.3, (58.9 -95.7)	>0.001
	Yes	7	126.3, (84.9 -231.6)	---	----------	
Total		119	126.6, (84.9 -231.6)	31	68.3, (58.9 -95.7)	<0.001

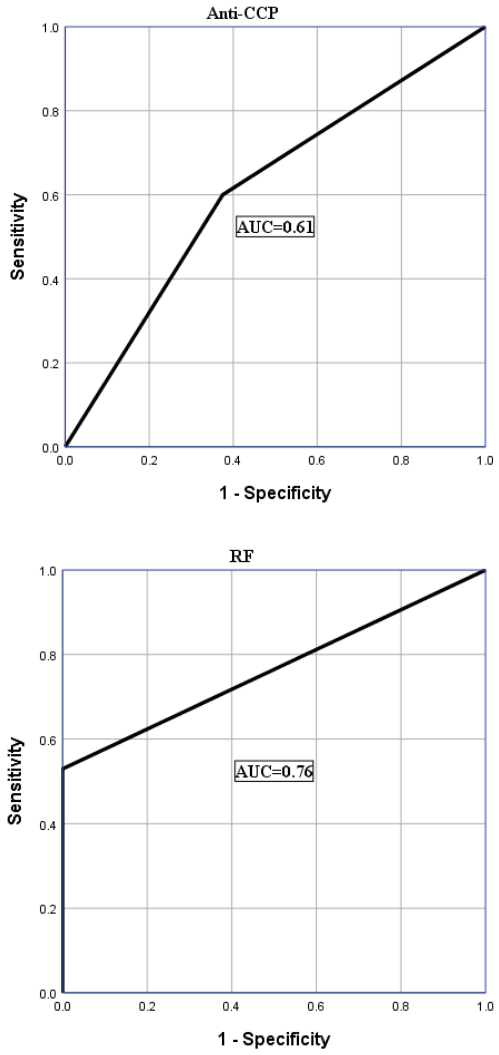
Rheumatoid factor (RF) and Anti cyclic citrullinated peptide (Anti-CCP) are the main autoantibodies in a RA patient. In Table 3 and figure 2 showed RF was the highest sensitivity (100.0%) and negative predictive value (100.0%), while anti-CCP specificity (60.0%) and positive predictive value (55.6%).

The area under the curve between RF and DAS-28 was (0.76); for anti-CCP (0.61), they were not predictive markers for DAS-28.

Table 3: Diagnostic properties of anti-CCP antibody and rheumatoid factor tests in rheumatoid arthritis with DAS-28.

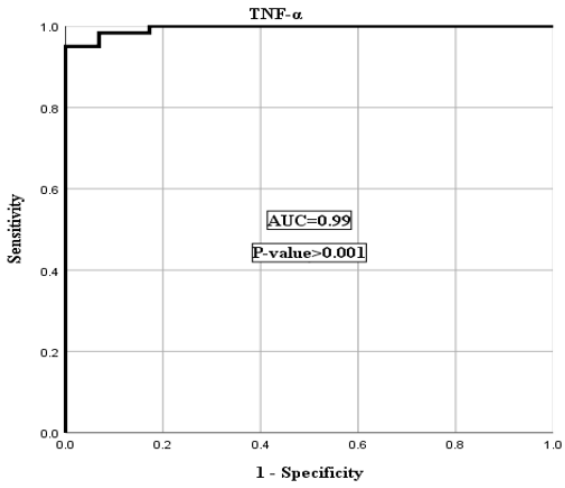
Test	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy
RF	100.0%	52.9%	20.0%	100.0%	57.9%
Anti-CCP	62.5%	60.0%	55.6%	66.7%	61.1%

Figure 2: Receiver operating curve (ROC) analysis of the RF and Anti-CCP for predicting DAS-28. AUC = area under the curve



The present study used ROC to evaluate the Area Under Curve (AUC), where AUC = 0.99 and P < 0.001. Indicating almost perfect discrimination or accuracy to predict disease development whenever AUC values become closer to one. The result showed a significant increase in cytokines in the patients' group compared to the control group, indicating an association between TNF- α and RA. (Fig. 3).

Figure 3: Receiver operating curve (ROC) analysis of the TNF-a for predicting rheumatoid arthritis. AUC = area under the curve.



Discussion

The pathogenesis of RA is complex; it involves several immune cells and the interaction between several cytokines that a single cytokine may not provide a sufficient

indication for rheumatoid arthritis. The recruitment and accumulation of immune cells are essential in rheumatoid arthritis (Riyadh Mohsen et al., 2021). Cytokines potentially reveal multi-types more advanced of rheumatoid arthritis (Chiad et al., 2015) and used as clinical biomarkers may represent a more accurate future of diagnostic medicine for rheumatoid arthritis the disease. At the same time, a complex combination of markers is needed to predict response to therapy(Wang et al., 2018). Demographic characteristics (sex, age, BMI, family history, smoker, duration of disease, and DAS-28) for patient and healthy groups show significance where p-value (0.001), and BMI (0.038). The relationship between sex hormones and RA led to differences between males and females in RA (Heikkilä et al., 1998). Generally, RA appears over the age of 40, but it can appear at different ages. It may also develop in children(Targońska-Stępniak et al., 2019). RA is more likely to appear in patients with family history than non. as a heterogenous disease; Smoking is considered one disease etiologies with BMI that significantly affects patients' rheumatoid arthritis(Prisco et al., 2020). Many cytokines are involved in early events in the rheumatoid synovium; this study demonstrated an increase in the level serum of TNF-α with RA patients compared to healthy individuals and showed significance in some demographics characteristics (age, BMI, and biological therapy) with TNF-α except. It results in a support characteristics study that found elevated TNF- α represents an inflammatory marker in rheumatoid arthritis patients(Moelants et al., 2013). Some therapies can reduce the activity level of Inflammatory etiology (Kanwal et al., 2013); biological therapy appears significant in reducing inflammation when compared to the TNF- α in two patient groups with treatment and un-treatment (Papadopoulos et al., 2005). ROC has been used to differentiate an individual's likelihood of developing rheumatoid arthritis. It was widely used in clinical epidemiology to diagnose various diseases, including autoimmune diseases like rheumatoid arthritis. Rheumatoid arthritis diagnosis by ROC analyses showed that TNF-α might be putative markers for RA. the evaluated RF and anti-CCP as a predictive factor for disease activity score 28 (Önder et al., 2009); it is still unknown if the extent of RF, Anti-CCP levels is helpful to assess the disease activity or the individual follow-up as an individual activity parameter. The regression estimation model for the RF and ACCP did not have any forecast for disease activity score 28 in RA patients. the results for RF and anti-CCP autoantibodies were not associated with scores of DAS-28(Karimifar et al., 2012). Consequently, further study is necessary to support the hypothesis that the anti-CCP antibody positivity is a risk factor in RA patients in larger sample sizes (Del Amo et al., 2006).

Conclusions

Level serum TNF- α in rheumatoid arthritis patients increased compared to healthy individual groups, but it's no significant correlation with demographic characteristics except age, BMI, and biologics therapy. Rheumatoid arthritis diagnostic by ROC analyses showed that TNF-α might be putative markers for disease. RF and anti-CCP in rheumatoid arthritis do not represent predicted indicators for DAS-28.

Conflict of Interest The authors declare that they have no conflict of Interest.

Acknowledgments

The medicine city Baghdad hospital \ and the consulting clinic Baghdad, Iraq, supported this

work. We appreciate the support of the University of Baghdad, the College of Sciences, and the Biotechnology Department.

Reference

ALETAHA, D., NEOGI, T., SILMAN, A. J., FUNOVITS, J., FELSON, D. T., BINGHAM, C. O., 3RD, BIRNBAUM, N. S., BURMESTER, G. R., BYKERK, V. P., COHEN, M. D., COMBE, B., COSTENBADER, K. H., DOUGADOS, M., EMERY, P., FERRACCIOLI, G., HAZES, J. M., HOBBS, K., HUIZINGA, T. W., KAVANAUGH, A., KAY, J., KVIEN, T. K., LAING, T., MEASE, P., MENARD, H. A., MORELAND, L. W., NADEN, R. L., PINCUS, T., SMOLEN, J. S., STANISLAWSKA-BIERNAT, E., SYMMONS, D., TAK, P. P., UPCHURCH, K. S., VENCOVSKY, J., WOLFE, F. & HAWKER, G. 2010. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum, 62, 2569-81.

AREND, W. P. & DAYER, J. M. 1995. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. Arthritis Rheum, 38, 151-60.

BURSKA, A., BOISSINOT, M. & PONCHEL, F. 2014. Cytokines as biomarkers in rheumatoid arthritis. Mediators Inflamm, 2014, 545493.

CHIAD, I. A., YOSSEF, H. S., AL-SAIDI, M. A. & ABBAS, M. A. 2015. Evaluation of Some Immunological Markers in the Rheumatoid Arthritis Patients. Iraqi Journal of Science, 56, 2488-2493.

DEL AMO, N. D. V., BOSCH, R. I., MANTECA, C. F., POLO, R. G. & CORTINA, E. L. 2006. Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. Clinical and experimental rheumatology, 24, 281-286.

FARRUGIA, M. & BARON, B. 2016. The role of TNF-α in rheumatoid arthritis: a focus on regulatory T cells. Journal of Clinical and Translational Research, 2.

HEIKKILÄ, R., AHO, K., HELIÖVAARA, M., KNEKT, P., REUNANEN, A., AROMAA, A., LEINO, A. & PALOSUO, T. 1998. Serum androgen-anabolic hormones and the risk of rheumatoid arthritis. Annals of the rheumatic diseases, 57, 281-285.

HUANG, C. C., CHIOU, C. H., LIU, S. C., HU, S. L., SU, C. M., TSAI, C. H. & TANG, C. H. 2019. Melatonin attenuates TNF‐α and IL‐1β expression in synovial fibroblasts and diminishes cartilage degradation: Implications for the treatment of rheumatoid arthritis. Journal of pineal research, 66, e12560.

INAM ILLAHI, M., AMJAD, S., ALAM, S. M., AHMED, S. T., FATIMA, M. & SHAHID, M. A. 2021. Serum Tumor Necrosis Factor-Alpha as a Competent Biomarker for Evaluation of Disease Activity in Early Rheumatoid Arthritis. Cureus, 13, e15314.

ISOMAKI, P. & PUNNONEN, J. 1997. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann Med, 29, 499-507.

KANWAL, N., JOHN, P. & BHATTI, A. 2013. MicroRNA-155 as a therapeutic target for inflammatory diseases. Rheumatol Int, 33, 557-60.

KARIMIFAR, M., SALESI, M. & FARAJZADEGAN, Z. 2012. The association of anti-CCP1 antibodies with disease activity score 28 (DAS-28) in

rheumatoid arthritis. Advanced biomedical research, 1.

KLARESKOG, L., RONNELID, J., SAEVARSDOTTIR, S., PADYUKOV, L. & ALFREDSSON, L. 2020. The importance of differences; On environment and its interactions with genes and immunity in the causation of rheumatoid arthritis. J Intern Med, 287, 514-533.

MCGONAGLE, D., WATAD, A. & SAVIC, S. 2018. Mechanistic immunological based classification of rheumatoid arthritis. Autoimmun Rev, 17, 1115-1123.

MOELANTS, E. A., MORTIER, A., VAN DAMME, J. & PROOST, P. 2013. Regulation of TNF‐α with a focus on rheumatoid arthritis. Immunology and cell biology, 91, 393-401.

ÖNDER, B., KURTARAN, A., KIMYON, S., SELÇUK, B. & AKYÜZ, M. 2009. Association of anti-CCP positivity with serum ferritin and DAS-28. Rheumatology international, 30, 223-227.

PALA, O., DIAZ, A., BLOMBERG, B. B. & FRASCA, D. 2018. B lymphocytes in rheumatoid arthritis and the effects of anti–TNF-α agents on B lymphocytes: a review of the literature. Clinical therapeutics, 40, 1034-1045.

PAPADOPOULOS, N., ALAMANOS, Y., VOULGARI, P., EPAGELIS, E., TSIFETAKI, N. & DROSOS, A. 2005. Does cigarette smoking influence disease expression, activity and severity in early rheumatoid arthritis patients? Clinical and experimental rheumatology, 23, 861.

PRISCO, L. C., MARTIN, L. W. & SPARKS, J. A. 2020. Inhalants other than personal cigarette smoking and risk for developing rheumatoid arthritis. Current opinion in rheumatology, 32, 279.

RIYADH MOHSEN, E., ALI, N. H. & ALDAOSERI, H. A. 2021. Immunologic Parameters for Disease Activity in Rheumatoid Arthritis. Arch Razi Inst, 76, 1095-1105.

SHRIVASTAVA, A. K., SINGH, H. V., RAIZADA, A., SINGH, S. K., PANDEY, A., SINGH, N., YADAV, D. S. & SHARMA, H. 2015. Inflammatory markers in patients with rheumatoid arthritis. Allergol Immunopathol (Madr), 43, 81-7.

TARGOŃSKA-STĘPNIAK, B., BISKUP, M., BISKUP, W. & MAJDAN, M. 2019. Gender differences in cardiovascular risk profile in rheumatoid arthritis patients with low disease activity. BioMed research international, 2019.

WANG, W., ZHOU, H. & LIU, L. 2018. Side effects of methotrexate therapy for rheumatoid arthritis: a systematic review. European journal of medicinal chemistry, 158, 502-516.

ZHANG, C. 2021. Flare-up of cytokines in rheumatoid arthritis and their role in triggering depression: Shared common function and their possible applications in treatment (Review). Biomed Rep, 14, 16.