Saadati N, et al. Iran J Immunol. 2020; 17(4):324-332.
https://doi.org/10.22034/iji.2020.83264.1617.

ORIGINAL ARTICLE

Serum Level of Soluble Lymphocyte-Activation Gene 3 Is Increased in Patients with Rheumatoid Arthritis

Nayyereh Saadati[1], Mandana Khodashahi[1], Zahra Rezaieyazdi[1], Maryam Sahebari[1], Zeinab Saremi[2]*, Saeed Mohammadian Haftcheshmeh[3], Houshang Rafatpaneh[1], Maryam Salehi[4]

[1]Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, [2]Internal Medicine Department, Faculty of Medicine, Birjand University of Medical Science, Birjand, [3]Department of Medical Immunology, Faculty of Medicine, [4]Center for Patient Safety and Clinical Research Unit, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Background: Rheumatoid arthritis (RA) is described as a systemic and chronic autoimmune disease characterized by inflammatory polyarthritis. Lymphocyte-activation gene 3 (LAG3) is a membrane glycoprotein expressed on activated, exhausted, and regulatory T cells. LAG3 plays a major role in the function of Treg cells. LAG3 also has a soluble form (sLAG3) with a controversial role. **Objective:** To evaluate the serum level of sLAG3 in rheumatoid arthritis patients in comparison with healthy subjects and assess its association with the disease activity. **Methods:** This cross-sectional study was performed on 105 patients with RA referred to Ghaem hospital of Mashhad, Iran. We divided the participants into four groups: 1) 35 untreated patients with newly diagnosed RA, 2) 35 active RA patients, 3) 35 patients in the remission phase of the disease, and 4) 35 healthy individuals matched in terms of age and sex. After completing the interview and questionnaire, the sLAG3 was evaluated by commercial ELISA. **Results:** The serum level of sLAG3 significantly increased in RA patients (76.78 ng/ml) as compared with the healthy participants (51.67, p=0.002). However, there was no significant difference between RA patients in the remission phase of the disease (114.11 ng/ml) and those with moderate to high disease activity (63.06 ng/ml, p=0.076). **Conclusion:** This study provided insights into the role of sLAG3 in the immunopathogenesis of RA disease, but further investigations are also warranted.

Received: 2019-09-14, Revised: 2020-10-22, Accepted: 2020-10-26.
Citation: Saadati N, Saremi Z, Khodashahi M, Rezaieyazdi Z, Sahebari M, Mohammadian Haftcheshmeh S, Rafatpaneh H, Salehi M. Serum Level of Soluble Lymphocyte-Activation Gene 3 Is Increased in Patients with Rheumatoid Arthritis. *Iran J Immunol.* 2020; 17(4):324-332. doi: 10.22034/iji.2020.83264.1617.

Keywords: Disease Activity, Regulatory T Cells, Rheumatoid Arthritis, Soluble LAG3

*Corresponding author: Dr. Zeinab Saremi, Department of Internal Medicine, Faculty of Medicine, Birjand University of Medical Science, Birjand, Iran, e-mail: zsaremi@bums.ac.ir

INTRODUCTION

As a chronic and systemic autoimmune disease, rheumatoid arthritis (RA) is defined by inflammatory polyarthritis, particularly inflammations in small joints (1,2). The prevalence of RA accounts for 0.5-1% of populations; (3) however, its etiology is not completely clear. RA results from a complex interaction between gene and environment. Furthermore, innate and adaptive immune system cells are recruited and accumulated along with the formation of villous projections of synovial tissue that can attack the cartilage and bone (4,5). Innate immune responses such as complement activation causes the initiation of inflammation in the synovia. Humoral adaptive immunity is an important player in the chronic inflammatory response; it generates anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF) and antibodies. The pathogenesis of RA is based on autoreactive T cells and abnormal thymic selection of T cell. Regulatory T cells (Tregs) are a group of T lymphocytes that inhibit the immune system. Tregs are directly released from the thymus, which contains 5-10% of a CD4 cell (6). They are important for the prevention of autoimmune diseases and the suppression of inflammation via the secretion of IL-10 and TGF-B and cell-to-cell contact mechanisms (7). Treg cells play an important role in the pathogenesis of RA and other autoimmune diseases, such as lupus erythematous (8). An imbalance between Tregs and pathogenic cells was observed in patients with RA (9). Research findings vary as to whether the number of circulating Tregs in patients with RA is greater than in healthy controls. However, the number of Tregs in the synovial fluid of RA patients was higher than in blood, despite high number of T regs in serum of RA patients, the suppresive function of these cells on immune activation, is impaired (10,11). Lymphocyte activation gene 3 (LAG3) protein is a candidate phenotypic surface marker for IL-10-producing Tregs (12-14). Nakachi *et al.* revealed that human $LAG3^+$ Tregs were more potent supressors of B-cell antibody production compared with $CD25^+$ Tregs in RA patients (12). Previous studies showed that LAG3 was required for the inhibitory mechanism of the Tregs. However, there is also a possibility that LAG3 can disrupt the function of Tregs. Zhang Q showed that mice lacking LAG3 T cells were less likely to develop autoimmune diabetes (15). The sLAG3 was produced from proteolytic cleavage of LAG3 by metalloproteinases; (16) also Yu Seri showed that in patients with systemic lupus erythematosus soluble LAG3, it was released from dendritic cells and increased in SLE patients (17). Pedersen J showed that soluble LAG3 increased in early RA patients with no correlation with disease activity (18). Therefore, the role of LAG3 in autoimmune disease is not completely obvious. The objective of this study was to compare the serum level of sLAG3 in RA and healthy participants and to assess its correlation with the disease activity of RA according to DAS28-ESR disease activity score.

MATERIALS AND METHODS

Patients. This cross-sectional study was conducted on 105 RA patients according to the American College of Rheumatology and European League Against Rheumatism 2010 (ACR/EULAR 2010). The appendix shows the criteria for the diagnosis of RA and DAS28 criteria. All RA patients who participated in this study were treated with prednisolone from 2.5 mg to 10 mg daily. Patients were divided into five groups based

on medication regimen: 1) only prednisolone, 2) prednisolone with sulfasalazine, 3) prednisolone with methotrexate, 4) triple therapy (prednisolone + sulfasalazine or hydroxycholoroquine + methotrexate), and 5) prednisolone + methotrexate and biologic agents including antiTNF alpha. We compared the serum level of *sLAG3* between different drug groups. The participants were patients referring to the Rheumatology Disease Research Center (RDRC) of Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, from November 2016 to December 2017. Healthy controls comprised 35 people referring to the Rheumatology Clinic of Ghaem Hospital during the research period. They complained about the nonspecific musculoskeletal symptoms without the diagnoses of RA or other rheumatologic disease. The patients were divided into three groups:1) untreated patients with newly diagnosed RA (35 patients), 2) 35 active RA patients, and 3) 35 patients in remission phase. Disease activity was specified based on the DAS28 ESR criteria in the Appendix. Patients with active disease had DAS28 ESR>3.2 and those in the remission phase had DAS28 ESR<2.6. The Disease Activity Score 28-joint count (DAS28) scores were numerated using erythrocyte sedimentation rate (ESR). The recommendations of American College of Rheumatology (ACR) on the use of DAS28-CRP or DAS28-ESR were considered to determine the disease activity of RA. Of note, the study participants were matched for age and sex. The exclusion criteria were heart failure, renal or hepatic failure, malignancies, pregnancy, overlap syndromes, and collagen vascular diseases (systemic lupus erythematosus, primary Sjögren's syndrome, systemic scleroderma, and inflammatory myopathy) alcohol usage, infections, and other autoimmune diseases. All the patients and healthy controls underwent physical examination by a rheumatologist. After the participants completed the questionnaire and signed the consent form, 10 cc blood sample was taken. Afterwards, the serum samples were separated through centrifugation, transferred to the refrigerator, and kept at a temperature of -20°C (it took eight months to collect all samples).

ELISA. Sandwich enzyme-linked immunosorbent assay was used to detect the concentration of *sLAG3* for each sample. The following kit was used to test soluble LAG3 kit (ZellBio GmbH, Germany, Cat No: ZB-15103C-H9648) with a normal range of 20-640 ng/ml. Each patient sample was checked two times by ELISA. Finally, the serum sLAG3 level of the healthy subjects was compared with the RA patients. Moreover, the cases with RA were compared in terms of disease activity and treatment type.

Ethical Considerations. The research method was authorized by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.fm.REC.1396.149). Informed consent form was obtained from all study participants, and they were assured that their data would remain private. They were allowed to withdraw from the study at any time.

Statistical Analysis. The descriptive data were analyzed using SPSS software (version 16). To evaluate the normality of data, the Kolmogorov-Smirnov test was utilized. The qualitative variables were analyzed using the Chi-square tests and quantitative variables by t-test. Data were expressed as mean ± SD. p-values<0.05 were considered as statistically significant.

RESULTS

The mean age of the participants was 49.86 ± 11.08 years. About 10.71% (n=15) and 89.29% (n=125) of the patients were male and female, respectively. Table 1 shows the frequency of demographic information in the four groups. No remarkable difference was observed in the sex distribution among the four groups (p=0.87). The age distribution was not different among groups (p=0.17).

Table 1. Frequency of demographic information in four groups.

Variable		Controls (n=35)		RA remission (n=35)		Newly RA (n=35)		Active RA (n=35)		p-value
		Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Age		51.31	10.54	48.11	11.76	48.94	13.31	51.88	1.01	0. 17
Age		51.31	10.54	49.31 ± 12.1						0.584
		%		%		%		%		
Gender	Male	11.43 (n=4)		11.43 (n=4)		8.57 (n=3)		11.43 (n=4)		0.87
	Female	88.57 (n=31)		88.57 (n=31)		91.43 (n=32)		88.57 (n=31)		

There was no significant different between RA (49.31 ± 12.1) and the controls (51.31 ± 10.54) in terms of age (p=0.584). The disease duration in the active RA group was less than one year in 8.7%, between 1-5 years in 31.4%, and more than 5 years in 60%. Moreover, in patients in the remission phase of the disease, this duration was less than 1 year in 20%, 1-5 years in 34.28%, and more than 5 years in 45.71%. There was no significant difference between the active (35 patients) and remission groups (35 patients) in terms of disease duration. Six cases (5.71%) with RA had extra-articular organ involvement, of which five patients (83.34%) were in the active RA group. Furthermore, among subjects with extra-articular organ involvement, four participants suffered from interstitial lung disease, one had scleritis, and one patient had co-existing lung and eye in the form of scleromalacia. The serum concentration of sLAG3 significantly increased in RA patients (76.78 ± 32.12 ng/ml) compared with the healthy controls (51.67 ± 28.5 ng/ml) (p=0.002). The assessment of the four groups in terms of sLAG3 demonstrated that the mean of sLAG3 concentration was 51.67 ng/ml ± 28.5 in the control group, 114 ng/ml ± 39.2 in RA patients in remission phase, 54 ng/ml ± 15.17 in those newly diagnosed with RA, and 63 ng/ml ± 35.7 in active RA patients (Table 2).

Table 2. Comparison of sLAG3 values by groups by *t test*.

Variable	Active RA (n=35)	New Case RA (n=35)	Remission RA (n=35)	Healthy Control (n=35)	p-value
sLAG3	63 ± 35.7	54 ± 15.17	114 ± 39.2	52 ± 28.5	**0.001<**

The serum concentration of sLAG3 among the newly diagnosed RA patients was significantly lower than active RA patients (p=0.03), as well as in RA new cases lower

than patients in remission phase of RA (p<0.001), (Table 3). However, no significant difference was found between the newly diagnosed RA and the healthy controls (p=0.97), active RA patients and healthy individuals (p=0.18), and active RA patients and remission groups (p=0.076). Table 4 shows the serum concentration of sLAG3 based on DAS28 ESR. American College of Rheumatology (ACR) has approved a list of disease activity scales that have been demonstrated its relation with outcome. One of the most common disease activity scales is DAS 28 ESR.

Table 3. Intra-group comparison of sLAG3 values in groups by Dent 3 test.

Group	Compared with	p-value
New RA	Active RA	0.035
	Healthy Control	0.975
	Remission RA	<0.001
Active RA	Remission RA	0.076
	Healthy control	0.18
Remission RA	Healthy control	0.005

Based on DAS28 ESR, RA patients were divided into four groups: ≤2.6 means remission (47 patients), 2.6-3.2 show a low disease activity (1 patient), > 3.2 and ≤5.1 refer to a moderate disease activity (32 patients), and >5.1 indicate a high disease activity (25 patients).

Table 4. Serum concentration of soluble LAG 3 based on DAS 28 ESR scale. Kruskal-Wallis followed by Dunn's multiple comparisons test was used for analysis.

Variable		Median	Range		p-value
			Minimum	Maximum	
Disease Activity Score 28	*5.1<	52	43.5	61.5	<0.001
	5.1-3.2	67.5	54.3	88	
	3.2-2.6	65	65		
	*≤2.6	89	58	139.5	

* Difference is between these groups. Results are express as median (range).

Further analysis showed that patients in remission phase (DAS 28 ESR≤2.6) had a higher level of sLAG3 compared to those with high disease activity scores (DAS 28 ESR. >5.1) (p<0.001). Further analysis indicated a modest correlation coefficient between DAS28-ESR and sLAG3 (r=-0.43). Analysis of sLAG3 concentration in different drug regimens showed that there was no significant difference between patients under different drug regimens (p=0.33). Based on the disease duration, RA patients were divided into three groups, including <1 year, 1-5 years, and >5 years. The obtained results of K2 test revealed no significant difference between the active RA and remission RA regarding the disease duration (p>0.05). There was no significant difference between sLAG3 serum level and age and sex parameters. A t-test was used to examine the relationship between the sex and serum level of sLAG3 (p=0.17). A

correlation test was used to examine the relationship between age and serum level of sLAG3 with r=0.16 and p=0.06. Therefore, age, sex, drug use, and disease duration, as confounding factors, did not affect the serum sLAG3 level.

DISCUSSION

Rheumatoid arthritis (RA) is one of the most prevalent chronic diseases with an immune-based process characterized by the uncontrolled inflammatory responses due to the breakdown of self-tolerance (19). Unabated inflammatory responses mediated by several inflammatory immune cells are among the major causes of RA (20). Previous studies have noted the importance of sLAG3 in the pathogenesis of several diseases (17,21,22). This study sought to determine the serum concentration sLAG3 in RA patients. We found that the serum level of sLAG3 in patients with RA was significantly higher than that of the healthy controls. Moreover, the serum level of sLAG3 was different among the newly diagnosed subjects, patients with active RA, and patients in the remission phase. However, there was no significant difference in the serum level of sLAG3 in RA patients in remission phase patients compared to those with moderate to severe disease activity. As a membrane form, LAG3 was expressed on the surface of activated T CD4 and T CD8 cells, Treg cells, and B lymphocytes. Membrane LAG3 has negative regulatory roles in the proliferation, activation, and function of CD4+ and CD8+ T cells. Interestingly, expression of LAG3 on cell surface is regulated by two important metalloproteases, namelyADAM10 and ADAM17 (16,23,24). These metalloproteases cleave the membrane form of LAG3 at the site of the connecting peptide in the extracellular portion, resulting in the formation of its monomeric soluble form (25). Previous research has demonstrated that sLAG-3 has immune adjuvant activity. Moreover, growing evidence indicates that sLAG3 can be considered as a marker of TH1 differentiation and activation (26). sLAG3 can also compete with the membrane LAG3 on the surface of Treg cells and limit the negative regulatory functions of LAG3 (16). In a study conducted by Yu *et al.*, it was shown that serum concentration of sLAG3 significantly increased in patients with SLE compared to the healthy controls (17). sLAG3 exerts immune adjuvant activity and induces the activation and maturation of dendritic cells (DCs), leading to the priming of auto-reactive T cells, especially TH1 cells, and promoting inflammatory responses in SLE (17). These results support our findings and indicate that sLAG3 can be considered as a specific, novel marker for autoimmune diseases such as RA and SLE. Several research reports have recently demonstrated the potential roles of sLAG3 in the pathogenesis and development of autoimmune diseases. In this regard, Zhang Q *et al.* revealed that sLAG3 limited the proliferation and function of Treg cells in autoimmune diabetes. By producing several anti-inflammatory cytokines such as IL-10, Treg cells play key roles in controlling the inflammatory responses (27). Therefore, by inhibiting the proliferation and function of these suppressor cells, sLAG3 can induce autoimmunity (15). In a study conducted by Pedersen J *et al.* increased levels of sLAG3 were observed in early RA patients (18). In this regard, our results confirmed the findings of a previous study. We found higher concentrations of sLAG3 in RA patients compared to healthy controls. This can be explained in part by the fact that the majority of TH1 cells release sLAG3, which is

positively associated with the production of IFN-γ (21). TH1 cells are mainly responsible for Type 4 hypersensitivity through producing several pro-inflammatory cytokines such as TNF and IFN-γ, which are widely associated with multiple inflammatory and autoimmune diseases like RA (28-31). In accordance with the results of Pedersen J, in which no correlation was found between the plasma levels of sLAG3 and disease activity, the serum levels of sLAG3 were not higher in patients with high to moderate disease activity than patients with remission phase in our study. It seems that sLAG3 plays a crucial role in the pathogenesis of RA; however, the association of serum sLAG3 with disease activity is yet to be clarified. The low number of participants and the brief period of follow-up were the main constraints of this study. Accordingly, our results cannot be generalized to other people. More studies with larger sample sizes must be carried out to obtain more accurate results. Moreover, it is recommended that more research be done with longer follow-up periods to evaluate the potential roles of sLAG3 in the pathogenesis of RA. Furthermore, more studies need to be performed on the effect of drugs in the serum concentration of sLAG3 in RA patients. In conclusion, our results showed that the serum concentration of sLAG3 significantly increased in RA patients as compared to the healthy participants. These findings contribute to the research into the probable roles of sLAG3 in the immunopathogenesis of RA disease. Based on our findings, sLAG3 can be considered as a specific, novel marker for autoimmune diseases such as RA.

ACKNOWLEDGEMENTS

This study was conducted by Rheumatic Diseases Research Center of Mashhad University of Medical Sciences, Mashhad, Iran (Grant No. 951602).

REFERENCES

1. Smolen JS, Aletaha D, Redlich K. The pathogenesis of rheumatoid arthritis: new insights from old clinical data? Nat Rev Rheumatol. 2012; 8:235-43.
2. Saghafi M, Khodashahi M, Saadati N, Azarian A, Rezaieyazdi Z, Salehi M, et al. Relationship between cartilage oligomeric matrix protein (COMP) and rheumatoid arthritis severity. Electron Physician. 2017; 9:5940-47.
3. Cross M, Smith E, Hoy D, Carmona L, Wolfe F, Vos T, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Annals of the rheumatic diseases. 2014; 73:1316-22.
4. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365:2205-19.
5. Esensten JH, Wofsy D, Bluestone JA. Regulatory T cells as therapeutic targets in rheumatoid arthritis. Nat Rev Rheumatol. 2009; 5:560-5.
6. Kurose K, Ohue Y, Wada H, Iida S, Ishida T, Kojima T, et al. Phase Ia Study of FoxP3+ CD4 Treg Depletion by Infusion of a Humanized Anti-CCR4 Antibody, KW-0761, in Cancer Patients. Clin Cancer Res. 2015; 21:4327-36.
7. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365:2205-19.
8. Iikuni N, Lourenço EV, Hahn BH, La Cava A. Cutting edge: regulatory T cells directly suppress B cells in systemic lupus erythematosus. J Immunol. 2009; 183:1518-22.

9. Boissier MC, Assier E, Biton J, Denys A, Falgarone G, Bessis N. Regulatory T cells (Treg) in rheumatoid arthritis. Joint Bone Spine. 2009; 76:10-4.
10. van Amelsfort JM, Jacobs KM, Bijlsma JW, Lafeber FP, Taams LS. CD4+ CD25+ regulatory T cells in rheumatoid arthritis: differences in the presence, phenotype, and function between peripheral blood and synovial fluid. Arthritis Rheum. 2004; 50:2775-85.
11. Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J Exp Med. 2004; 200:277-85.
12. Nakachi S, Sumitomo S, Tsuchida Y, Tsuchiya H, Kono M, Kato R, et al. Interleukin-10-producing LAG3+ regulatory T cells are associated with disease activity and abatacept treatment in rheumatoid arthritis. Arthritis Res Ther. 2017; 19:97.
13. Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nat Med. 2013; 19:739-46.
14. Okamura T, Sumitomo S, Morita K, Iwasaki Y, Inoue M, Nakachi S, Komai T, Shoda H, Miyazaki JI, Fujio K, Yamamoto K. TGF-β3-expressing CD4+ CD25− LAG3+ regulatory T cells control humoral immune responses. Nat Commun. 2015; 6:6329.
15. Zhang Q, Chikina M, Szymczak-Workman AL, Horne W, Kolls JK, Vignali KM, et al. LAG-3 limits regulatory T cell proliferation and function in autoimmune diabetes. Sci Immunol. 2017; 2:eaah4569.
16. Li N, Wang Y, Forbes K, Vignali KM, Heale BS, Saftig P, et al. Metalloproteases regulate T-cell proliferation and effector function via LAG-3. EMBO J. 2007; 26:494-504.
17. Yu S, Fujio K, Ishigaki K, Shoda H, Okamura T, Noor T, et al. Increased concentration of serum soluble LAG3 in systemic lupus erythematosus. Arthritis Res Ther. 2012; 14(Suppl 1): P16.
18. Pedersen JM, Hansen A, Hvid M, Horslev-Petersen K, Hetland ML, Stengaard-Pedersen K, et al., editors. Lymphocyte Activation Gene 3 Plasma Level Is Increased and Associated with Progression in Early Rheumatoid Arthritis: A Population-Based Cohort Study from Danbio and the Danish National Patient Registry. The American College of Rheumatology, ACR/ARHP Annual Meeting; 2018. 1; 70(Suppl. 9):141.
19. Firestein GS, McInnes IB. Immunopathogenesis of Rheumatoid Arthritis. Immunity. 2017; 46:183-96.
20. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med. 2001; 344:907-16.
21. Annunziato F, Manetti R, Tomasévic I, Guidizi MG, Biagiotti R, Giannò V, et al. Expression and release of LAG-3-encoded protein by human CD4+ T cells are associated with IFN-gamma production. FASEB J. 1996; 10:769-76.
22. Lienhardt C, Azzurri A, Amedei A, Fielding K, Sillah J, Sow OY, et al. Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. Eur J Immunol. 2002; 32:1605-13.
23. Chen D-Y, Chen Y-M, Chen H-H, Hsieh C-W, Lin C-C, Lan J-L. Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF-α therapy. Arthritis Res Ther. 2011; 13:R126.
24. Huard B, Mastrangeli R, Prigent P, Bruniquel D, Donini S, El-Tayar N, et al. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. Proc Natl Acad Sci USA. 1997; 94:5744-9.
25. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. Curr Top Microbiol Immunol. 2011; 344:269-78.
26. Annunziato F, Manetti R, Tomasévic I, Giudizi MG, Biagiotti R, Giannò V, et al. Expression and release of LAG-3-encoded protein by human CD4+ T cells are associated with IFN-γ production. FASEB J. 1996; 10:769-76.
27. Malemud CJ. Defective T-cell apoptosis and T-regulatory cell dysfunction in rheumatoid arthritis. Cells. 2018; 7:223.
28. Powrie F, Coffman RL. Cytokine regulation of T-cell function: potential for therapeutic intervention. Trends Pharmacol Sci. 1993; 14:164-8.

29. Scott B, Liblau R, Degermann S, Marconi LA, Ogata L, Caton AJ, et al. A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. Immunity. 1994; 1:73-83.
30. Haftcheshmeh SM, Mohammadi A, Soltani A, Momtazi-Borojeni AA, Sattari M. Evaluation of STAT1 and Wnt5a gene expression in gingival tissues of patients with periodontal disease. J Cell Biochem. 2019; 120:1827-34.
31. Asadzadeh-Aghdaei H, Mashayekhi K, Koushki K, Azimzadeh P, Rostami-Nejad M, Amani D, et al. V617F-independent upregulation of JAK2 gene expression in patients with inflammatory bowel disease. J Cell Biochem. 2019; 120:15746-55.
32. Sidaway P. Breast cancer: LAG3 expression indicates favourable outcomes. Nat Rev Clin Oncol. 2017; 14:712.
33. Shapiro M, Herishanu Y, Katz BZ, Dezorella N, Sun C, Kay S, et al. Lymphocyte activation gene 3: a novel therapeutic target in chronic lymphocytic leukemia. Haematologica. 2017; 102:874-82.
34. Xia J, Ni Z, Wang J, Zhu S, Ye H. Overexpression of Lymphocyte Activation Gene-3 Inhibits Regulatory T Cell Responses in Osteoarthritis. DNA Cell Biol. 2017; 36:862-9.