

The Levels of mRNA for Pro- and Anti-inflammatory Cytokines and B Cell Amounts in Patients With Lupus Erythematosus

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Article History:

Received: November 8, 2022

Accepted: January 1, 2023

ePublished: March 30, 2023

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Abstract

Background: Systemic lupus erythematosus, is the most prevalent form of lupus. SLE is an immune system sickness wherein the resistant framework goes after its own tissues, causing boundless aggravation and tissue harm in the impacted organs. The purpose of this study was to examine the levels of mRNA for pro- and anti-inflammatory cytokines and B cell amounts in patients with lupus erythematosus.

Methods: This case-control study was performed on 6 patients of those with lupus who were higher than 18 years old and referred to Sina Hospital Clinic to be visited and examined during 2021-2022, and their lupus illness was definitely diagnosed by rheumatology and a dermatologist. The control and patient groups were analyzed using a paired *t* test.

Results: The results of the present study demonstrated that the number of B cells in the blood samples of the patients was lower than that of the control group. Based on the results, the levels of interleukin (IL)-17, IL-6, and IL-1 in the patient group were higher than in the control group ($P < 0.005$), indicating an increase in inflammation in these patients. Finally, the amount of inhibitory cytokine IL-10 was decreased in these patients.

Conclusion: Overall, pro-inflammatory cytokines were more secreted in patients with lupus and inhibitory cytokines were less; thus, cytokine can be used as a biomarker of inflammation in these patients.

Keywords: B-cell lymphocytes, mRNA, Lupus

Introduction

Lupus is a systemic and chronic autoimmune disease with significant heterogeneity in clinical symptoms, and the main cause of this disease remains unknown. However, the evidence indicates that lupus is a consequence of the intervention of environmental, immunological, and genetic factors, finally, causing a breakdown of tolerance toward self-antigens and the start of an abnormal immune response to them and tissue damage.¹⁻⁴ It seems that the basic defect in systematic lupus erythematosus (SLE) is the dysfunction of T lymphocytes in B lymphocyte control, leading to the activation of polyclonal B lymphocytes and the production of a large number of autoantibodies.⁵ This autoantibody can damage various tissues directly or as a result of immune complex deposition.⁶⁻⁸ The increase in the expression of inflammatory cytokines increases the proliferation of autoreactive B cells and the production of more autoantibodies. The overexpression of pro-inflammatory cytokines plays a role in the pathogenesis of lupus.⁹⁻¹² The excessive increase of interleukin (IL)-17 cytokine is also associated with an increase in the Th17 subset of T cells. IL-6 exerts an important role in B cell hyperactivity and immunopathology of SLE and may

directly contribute to mediating tissue damage. Based on these data, it was proposed that the inhibition of IL-6 in humans can improve systemic and local systemic inflammation in lupus.¹²⁻¹⁴ Accordingly, this study aimed to evaluate inflammatory and anti-inflammatory cytokines in lupus patients.

Materials and Methods

Patient and Cell Isolation

Overall, 24 samples were selected from lupus patients ($n = 12$) and healthy people ($n = 12$) as available sampling. Then, 5 cc of venous blood was taken from each patient. Monocyte cells were isolated and cultured by the peripheral blood mononuclear cell method. The mRNA was extracted with a TRIzol reagent based on the protocol. Patients suffering from lupus disease without treatment were chosen as the inclusion criteria. On the other hand, patients having a chronic disease or treated lupus were excluded from the study.

Real-time Polymerase Chain Reaction

The cDNA of the cells of the patients and the control group was diluted in a ratio of 1-6 using distilled water.



Primers were prepared to separately determine the expression levels of IL-17, IL10, IL-6, and IL-1 genes. The tube containing the material was centrifuged for a few seconds to thoroughly mix and homogenize the material. The cDNA was added to the wells, and the set time and temperature schedule for the RT-PCR included 10 minutes at 95°C (time and temperature of enzyme activation and the initial formation), 15 seconds at 95°C (time and temperature of each cycle), 60 seconds at 62°C (time and temperature of extension and annealing of each cycle), and reading of the irradiated fluorescent at the end of each cycle. The number of cycles was also 40 times. To increase accuracy, each reaction was performed in duplicate (for repetition). The primers are provided in Table 1.

Flow Cytometry

The isolated monocytes were washed with phosphate-buffered saline and added to flow cytometry tubes. The samples were divided into two parts called the test and isotype tubes. In addition, CD19 primary antibody was diluted and the IgGT-PE isotype was added to the isotype tube at a ratio of 1:100. Further, CD4 primary antibody was diluted and the IgGT-APC isotype was added to the isotype tube with a ratio of 1:50, and CD3 primary antibody diluted with a ratio of 1:100 was added. Incubation was performed at 4°C for 1 hour in the dark. Centrifugation was performed for 5 minutes at 1500 rpm, and diluted secondary antibody 488-IHouse anti-human Alexa was added to both test and isotype tubes at a ratio of 1:500. Incubation was conducted at 4°C for 1 hour in the dark, and after washing and centrifugation, it was read by flow cytometry and analyzed with FlowJo software, version 7.6.1.

Data Analysis

The obtained data were statistically analyzed by Excel and SPSS (version 16) software programs. $P > 0.05$ was considered significant. Quantitative information is presented as means \pm standard deviations (SD). One-

sample Kolmogorov-Smirnov test was used to check the normal distribution of quantitative data.

Results

Expression of Pro-inflammatory Cytokines IL-1, IL-17, IL-6, and IL-10 in the Monocytes of Lupus Patients Compared to the Control Group

The analysis of the level of IL-1 expression in 12 lupus monocyte cells and 12 normal monocyte cells represented a significant increase in IL-1 expression in patient samples compared to control samples ($P = 0.001$). The evaluation of the amount of IL-17 in 12 lupus samples and 12 normal samples with a t-test demonstrated a significant increase in IL-17 in patient samples in comparison to normal samples ($P < 0.001$). Examining the intensity of IL-6 expression revealed a significant increase in IL-6 expression in patient samples compared to the control samples ($P < 0.001$, Figures 1A, 1B, and 1C).

Based on the evaluation of the level of IL-10 expression, a significant decrease was found in IL-10 expression in patient samples compared to the control (Figure 1D).

The Results of B Cell Expression

According to data in Table 2, the number of B cells in the patient and normal blood samples was measured with flow cytometry and analyzed by a t-test, and the test results showed a significant increase in the expression of CD19 in lupus samples (Table 2).

Discussion

The role of proinflammatory cytokines in the pathogenesis of SLE is still controversial. Several studies investigated the cytokine profile of SLE patients in vivo and in vitro.¹⁵⁻¹⁷ However, the findings of studies in this area are contradictory. Cytokines are essential molecules involved in the differentiation, maturation, and activation of cells and thus have a naturally significant impact on the immune inflammatory response. In autoimmune diseases, cytokines may not only play a role in immune dysregulation but also in local inflammatory processes, ultimately leading to tissue destruction. Similar to the results of Brugos et al,¹⁸ Umar et al,¹⁹ and Cigni et al,²⁰ while contrary to present study results, the level of IL-1 in the SLE group was significantly higher than the control group in the current study. According to this finding, IL-1 can be used as an inflammatory marker of SLE disease activity. The results of our study are in line with those of Wong et al, Ouyang et al, Yang et al, Tang et al, and Zhao et al, demonstrating that the level of IL-17 in the patient group was higher than that of the control group, confirming an increase in inflammation in these patients.²¹⁻²⁶ However, other studies suggested that IL-6 serum levels may not be associated with SLE disease.²⁷⁻³⁰ Similar to the study by Uchida et al,³¹ and Yao et al,³² while contrary to the study by Ishida et al³³ and Abd Elazeem et al,³⁴ Wardowska et al,³⁵ and Liu et al,³⁶ the serum levels of IL-10 were significantly decreased in patients with SLE.

Table 1. The Primers of Real-Time Polymerase Chain Reaction

Primer		Sequence
IL-1 primer	F	3' AACCGGGTTTAGGGCTATG5'
	R	3' GAAAGGGCCCTTAACAATC5'
IL-17 primer	F	3' CCTTAGGTTGGGCTCTATG5'
	R	3' GAAAAGCCCTTTATGATC5'
IL-6 primer	F	3' CCCTAGGGTTTAGGGCTATG5'
	R	3' AAGGTAGCCCTTAACAATC5'
IL-10 primer	F	3' AACCGGTTCAATGGCTATG5'
	R	3' GAAAGCTAGGCCCTTAAATC5'
B-actin primer	F	3' ATTACCGGGTTGGGCTATG5'
	R	3' AATAGGGCCGTCACAATC5'

Note. IL: Interleukin.

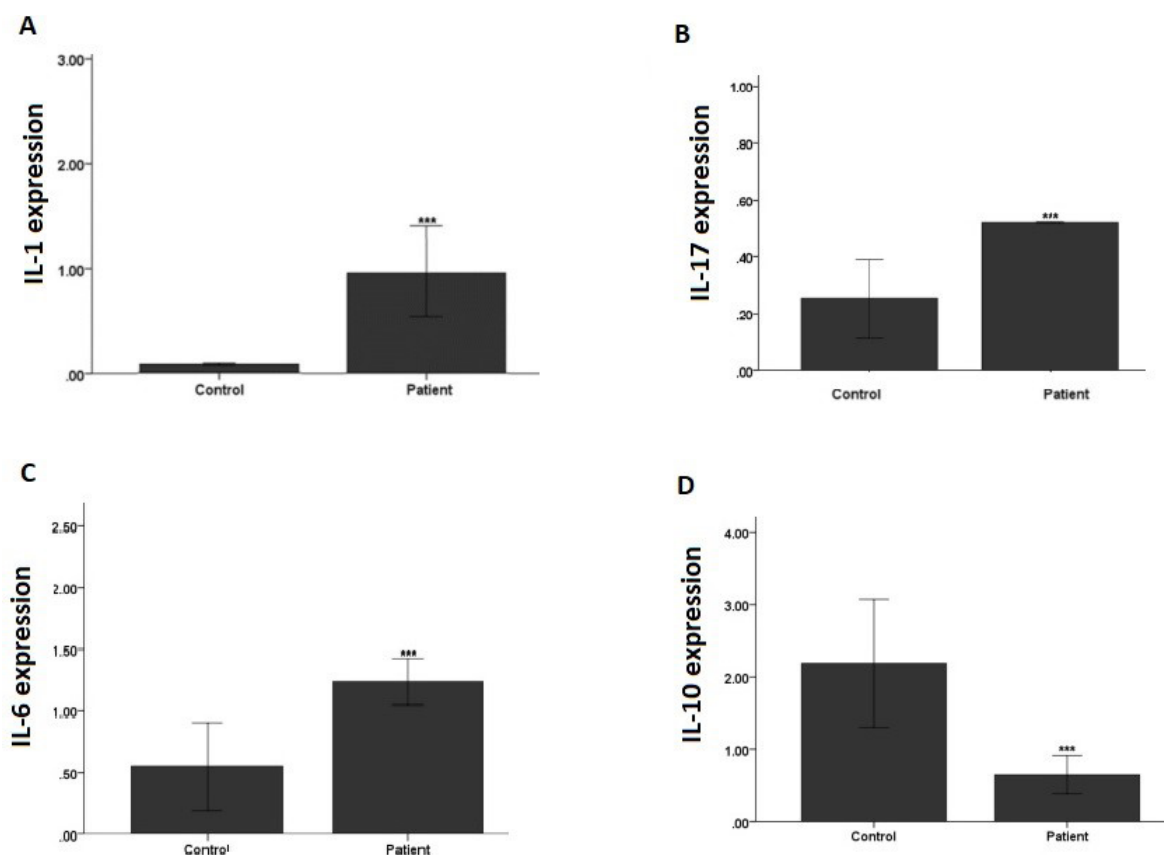


Figure 1. Cytokines Expression Levels in Systematic Lupus Erythematosus, (A) IL-1, (B) IL-17, (C) IL-6, and (D) IL-10. Note. IL: interleukin

Table 2. The B Cell Expression

	Normal	Patient
Mean \pm SD	2.4 \pm 10.08	2.01 \pm 26.8
P value	<0.005	<0.005

Note. SD: Standard deviation.

Our results conform to the findings of Korganow et al and Wardowska et al, representing that the expression of CD19, which was considered a B-cell diagnosis marker in the present study, was higher than that of the normal groups.^{35,37}

Conclusion

Our findings revealed that in terms of inflammation, pro-inflammatory cytokines IL-6, IL-17, and IL-1 had a significant increase in the patient group compared to the control group, but the level of IL-10 in the patient group was lower than the control group.

Acknowledgments

The authors would like to thank the Immunology Research Center of Tabriz University of Medical Sciences for cooperating in conducting the study.

Authors' Contribution

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Competing Interests

None.

Ethical Approval

The study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.633).

Funding

Self-funded.

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