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Determination of Expression Levels of Interleukin 1, 6, 17, 23 and Tumor Necrosis Factor Genes in Patients with Systemic Lupus Erythematosus

Kübra Solmaz[1], Eda Balkan[1*], Meltem Alkan Melikoğlu[3]

[1]Department of Medical Biology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Atatürk University, Erzurum, Turkey

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****Corresponding Author***
Eda Balkan
Department of Medical Biology
Faculty of Medicine
Atatürk University
Erzurum, Turkey.
Phone:+ +90 4423446946
E-mail: edadiyarbakir@atauni.edu.tr

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Authors' ORCIDs
Kübra Solmaz
http://orcid.org/0000-0002-3418-1896
Eda Balkan
http://orcid.org/0000-0002-7065-8161
Meltem Alkan Melikoğlu
http://orcid.org/0000-0001-7519-9470

Abstract: Systemic lupus erythematosus (SLE) is an autoimmune, chronic, and inflammatory disease. In many studies has done till these days, in patients with Systemic Lupus Erythematosus level of IL-1, IL-6, IL-17, IL-23, and TNF-α has been shown and these cytokines have a role in the pathogenesis of SLE disease. In our study, we aimed to detect the levels of these cytokines quantitatively. This study was carried out on 30 patients with SLE who were followed up in Erzurum Atatürk University Health Research and Application Center Physical Medicine and Rehabilitation Department Policlinic. Changes in mRNA expression of IL-1, IL-6, IL-17, IL-23, and TNF-α genes were determined by quantitative Real Time. There were no statistically significant differences between IL-6 gene expression levels between patient and control groups. There was a statistically significant difference between IL-1, IL-17, IL-23, and TNF- α genes between the patient and control groups. Although there are studies supporting the role of the IL-6 gene in the pathogenesis of SLE in the literature, in our study, there was no significant difference between SLE and control group. The threefold significant difference of the IL-1 gene between the SLE group and the control group supports the important role of the IL-1 gene in the pathogenesis of SLE. IL-17, IL-23, and TNF-α genes were also found to be significantly higher than SLE patient control groups. We think that these genes will contribute to clarifying the inflammation events in the etiopathogenesis of this disease. ©2023 NTMS.

Keywords: Systemic Lupus Erythematosus; Cytokines; Pathogenesis.

1. Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune, multisystemic, inflammatory connective tissue disease characterized by a variable course and prognosis of unknown etiology [1]. Although its etiology is not fully understood, it is known that genetic predisposition and environmental triggers cause Systemic Lupus Erythematosus (SLE). Systemic Lupus Erythematosus (SLE) is one of the most common autoimmune disorders affecting women of all age groups. It mostly affects women of childbearing age and is the age group in which the incidence peaks [2]. According to the genders, the prevalence was stated to be 9/1 between female/male. In addition, the incidence of Systemic Lupus Erythematosus (SLE), which differs according to age, decreases to 2/1 in the elderly and children [3]. Systemic Lupus Erythematosus (SLE) with

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a worldwide prevalence of 4.9/100.000 may show different incidence in different regions and geographical conditions[4]. Gender, ethnicity, sun exposure, age, and many genetic and environmental factors are the most influential factors in incidence and prevalence. In the United States (USA), the overall incidence is between 1.6 and 7.6 per 100,000, and the prevalence is between 14.6 and 508 per 100.000 is considered [5].

In the literature review, in animal model study of Edberg JC et al., PDCD1, known as the programmed cell death 1 gene, was reported to be associated with Systemic Lupus Erythematosus (SLE). Regarding the acute phase protein (CRP), a single nucleotide polymorphism study of the CRP gene rs3093061 was performed on patients with SLE and a correlation was found between the sensitivity of the CRP gene to Systemic Lupus Erythematosus (SLE) [6].

ITGAM (CD11b), another gene shown to be associated with Systemic Lupus Erythematosus (SLE), obtained statistically significant results in terms of SLE risk of ITGAM rs1143679 gene variants in patients with Systemic Lupus Erythematosus (SLE) of European origin.

According to data obtained from field studies, there are more than 100 gene polymorphisms that contribute to Systemic Lupus Erythematosus (SLE) susceptibility [7]. Recent research has focused on TOLL- like receptors (TLRs). Type I interferons have focused on variants of TLRs involved in immune system regulation pathways. Also, in studies on gene expression, TLR7 overexpression has been observed in patients with Systemic Lupus Erythematosus (SLE) [7].

2. Material and Methods

2.1. Materials

A patient group consisting of 30 patients (16 females-14 males) diagnosed with SLE and followed in Erzurum Atatürk University Health Research and Application Center Directorate Physical Medicine and Rehabilitation Department Polyclinic and 20 healthy individuals (10 females-10 males) without any systemic disease was included in our study. Informed Consent Form was signed by the patient and control group who agreed to participate in the study.

Blood samples collected for this study, which was approved by Erzurum Atatürk University Faculty of Medicine Ethics Committee, were used for RNA isolation (Roche) and gene expression studies (Roche Light Cycler 480 Real-Time) in Atatürk University Faculty of Medicine Laboratory of Medical Biology Department.

2.2. Methods

2.2.1. Gene expression analysis

2 ml EDTA blood samples were taken from 30 patients diagnosed with SLE and 20 control groups in Erzurum Atatürk University Health Research and Application Center Directorate.

Commercial isolation kit (High Pure) was used to synthesize cDNA from these blood samples taken from the patients of the Physical Medicine and Rehabilitation Department Polyclinic. Total RNA was obtained using RNA Isolation Kit-Roche) and stored in a deep freezer at -80 °C throughout the study. The procedure and chemicals recommended by the manufacturer were used for isolation.

The amount and quality of the obtained RNA were measured spectrophotometrically using the Nanodrop device (MaestroNano-USA). Measurement was made with 2 µl of RNA sample. Measurements made with the spectrophotometer at two wavelengths for quality; It has values between 260/280=1.5-1.9.

2.2.2. cDNA Synthesis

Commercial kit (ProtoScript® II First Strand cDNA Synthesis Kit, NEB) was used for cDNA synthesis from isolated mRNAs. In the synthesis process, the protocols determined by the manufacturer were taken as basis. The obtained cDNAs were stored at -20 ºC for use in the Real Time stage.

2.2.3. Statistical analysis

SPSS 20.0 (Statistical Packages for the Social Sciences for Windows XP Release 20.0 version) program was used to evaluate all statistical data related to the study. A Chi-square test was performed on the patient and control groups. Statistically significant differences are presented as follows: $p>0.05$ (not significant, ns) and $p<0.05$ (significant).

3. Results

A patient group consisting of 30 patients (16 females - 14 males) diagnosed with Systemic Lupus Erythematosus (SLE) and the control group consisting of 20 healthy individuals (10 females-10 males) without any systemic disease were included in this study. Expression levels of interleukin 1-6 tumor necrosis factor interleukin 17 interleukin 23 genes were examined.

Table 1: Patient and control group gene expression levels.

Gene symbol	Patient	Control	Value
IL-1	22.821	6.836	P<0.001
IL-6	11.399	10.573	P>0.05
IL-17	19.002	10.088	P<0.05
IL-23	20.062	11.392	P<0.05
TNF-α	19.554	9.914	P<0.05

When the IL-1 expression levels of the control and patient groups were evaluated, a 3- fold difference was found statistically. When the IL-6 levels of the control and patient groups were evaluated, no statistically significant difference was found. A 2-fold difference was observed in the IL-17 expression level analysis of the control and patient groups. A significant difference

was observed in the determined IL-23 expression levels of the control and patient groups.

4. Discussion

Systemic Lupus Erythematosus (SLE) is a multisystemic connective tissue disease characterized by variable course and prognosis [8]. Although complex genetic diseases and environmental factors are at the root of SLE, the exact cause of autoimmunity is unknown [9]. Studies using the MRL/lpr mouse model in the investigation of lupus and similar autoimmune diseases have reported that increased IL-1β gene expression is associated with disease severity and rapid progression of the disease. In the studies carried out to date, no investigation of the expression level of the IL-1 gene was found in Systemic Lupus Erythematosus (SLE) patients, only the serum level was examined. The study containing the most important data was conducted by Rachel M in 2018 and focused on serum levels in SLE patients [10]. In the study conducted by Hye-Young Chun in 2007, IL-2, IL-6, IL-10, IL-12 expression levels were examined, and no significant difference was observed in the IL-2 gene, while IL-10, IL-12, IL-6 genes were significantly different compared to the control group has been observed to increase [11]. Likewise, in the study of Birner P, an increase in IL-6 mRNA expression level was observed in Systemic Lupus Erythematosus (SLE) patients, and a statistically significant difference was obtained [12]. In our study, IL-6 gene expression was compared between the Systemic Lupus Erythematosus (SLE) patient and control groups, and the expression level of the said gene was not found to be statistically significant (P >0.05). In the study focused on investigating the relationship between IL-17 and T cell, conducted by Chun Kwok Wong in 2018, the expression levels were also examined, and a significant difference was found in the comparison of the expression level between the patient and control group, as in our study [13]. Tekin BG. investigated the relationship between vitamin D metabolism and interleukin 17 serum levels in SLE patients. If the study included the other, no difference could be found between the patient and the control group[14]. Likewise, in 2010, Keskin O. prepared the IL17 mRNA expression supplementary study of IL-17.IL-23 genes, and their effectiveness between the patient and control group IL-17 expression pressure no difference was found[15]. Dong G, in his study conducted in 2003, supports our study, and the difference between patient control and expression levels was found to be twofold and significant [16]. In our study, the expression levels of IL-17 genes of Systemic Lupus Erythematosus (SLE) patients were examined. The IL-17 expression level of the Systemic Lupus Erythematosus (SLE) group showed a significant 2-fold increase compared to the control group (P<0.05).

Roba M. Talaat, in his study to investigate the cytokine secretion profile in Systemic Lupus Erythematosus (SLE) patients and their possible relationship with disease activity, found a significant difference in IL-23 expression level [17].Another study parallel to our study was conducted by Xinfang Huang in 2014, and statistically significant results were obtained in this study, as in our study [18]. In addition, a statistically significant difference was found in the expression level of TNF-α gene in the study of Sabry et al.[19].

In the study, the expression levels of IL-1 genes of SLE patients were examined, IL-1 expression level of the Systemic Lupus Erythematosus (SLE) patient group increased significantly 3 times compared to the control group (P <0.001). In our study, IL-6 gene expression was compared between the Systemic Lupus Erythematosus (SLE) patient and control groups, and the expression level of the said gene was not found to be statistically significant (P>0.05). Expression levels of IL-17 genes of SLE patients were examined. Expression levels of IL-23 genes of Systemic Lupus Erythematosus (SLE) patients were examined. IL-23 expression level of the SLE patient group showed a significant increase compared to the control group (P<0.05). Expression levels of TNF-α genes of Systemic Lupus Erythematosus (SLE) patients were also examined. TNF-α expression level of the SLE patient group showed a significant increase compared to the control group (P<0.05).

5. Conclusions

As a result, according to the results of Real time PCR quantitative analysis, no statistically significant difference was observed between the patient and control groups in the expression levels of the IL-6 gene. It was observed that there was a statistical difference between the patient and control groups in IL-1, IL-17, IL-23 and TNF-α genes

Limitations of the Study

There are two major limitations in this study that can be addressed in future research. First, the sample size is larger. Second, showing that the number of interleukins involved in sle disease is higher.

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Conflict of Interests

The authors declare that there is no potential conflict of interest for the research, authorship, and/or publication of this article. All authors read and approved the final manuscript.

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Author Contributions

Design of the study: EB, Sample collection: MAM, Performed the experiments: KS, Data Collection and/or Processing: EB, KS, Writing Original Manuscript: EB, KS. EB contributed to revising the work and final approval of the final version of the manuscript..

Ethical Approval

This study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (05/01-07.06.2018).

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author.

Consent to participate

Consent was obtained from the patient and control groups participating in the study.

Informed Statement

The patient and control group who agreed to participate in the study signed the informed consent form.

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