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ORIGINAL ARTICLE

Evaluation of the relationship between IL-10, IL-17, IL-23 levels and disease activity of systemic lupus erythematosus and vitamin D status

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Abstract

Systemic lupus erythematosus (SLE) is a multisystemic, autoimmune connective tissue disease with a variable course and prognosis. We intended to determine IL-10, IL-17 and IL-23 cytokines and vitamin D levels in SLE patients, which we think play role in the pathogenesis of the disease. Forty SLE patients and 20 healthy controls were included in our study. Levels of IL-10, IL-17 and IL-23 were measured by sandwich ELISA method. Quantitative data are expressed as mean ± Standard deviation and median range (maximum-minimum) values. The data were analyzed at 95% confidence interval, and cases where the p value was less than 0.05 were considered statistically significant. IL-10 and IL-17 levels of the control and patient groups were compared and no significant difference was found (p=0.333, p=0.99). IL-23 levels of the patient group were found to be higher than the control group and were found to be statistically significant (p<0.001). No significant relationship was found between disease duration or SLEDAI score and IL-23 levels(p=0.476). 25 (OH) vitamin D levels of the patient group were found to be lower than the control group and were statistically significant (p=0.003). No significant relationship was found between IL-10 and IL-17 levels and vitamin D. Significant relationship was found between IL-23 and vitamin D levels (p=0.019). In our study, there was no significant difference between the groups in terms of IL-10 or IL-17, while IL-23 levels were found to be significantly higher in SLE patients. Vitamin D levels were found to be lower in the patient group with SLE compared to the control group, and a negative correlation was found between the disease duration and IL-23. Specific blocking of the IL-23 immune pathway can be an effective and safe treatment option in the treatment of SLE.

Keywords: *Systemic lupus erythematosus, IL-10, IL-17, IL-23, Vitamin D.*

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Introduction

Systemic lupus erythematosus (SLE) is a multisystemic, autoimmune, inflammatory disease with different laboratory and clinical features, characterized by a variable course and prognosis. Although the etiopathogenesis of the disease is not known exactly, genetic factors are the strongest determinants of the disease. Chemical substances, hormonal and environmental factors are other reasons that trigger the disease. As a result of abnormal regulation of T cells, events such as impaired immune tolerance, abnormal response to autoantigens, abnormal signal transmission between T cell receptors also contribute to SLE autoimmunity [1]. Interleukin-17 (IL-17) is an inflammatory cytokine, derived from Th 17 cells that has many functions in the regulation of tissue inflammation, B lymphocyte proliferation and antibody secretion in SLE patients [2]. Interleukin-23 (IL-23) molecule is needed in the stabilization and development of Th17 cells. IL-23 is predominantly secreted by antigen presenting dendritic cells and macrophages [3]. Interleukin-10 (IL-10) has positive effects on B cell differentiation, proliferation and autoantibody formation, and dysregulation in this cytokine is thought to be associated with many infectious and autoimmune diseases, including SLE [4]. Vitamin D is a hormone in steroid structure. In addition to calcium and bone metabolism, it also has effects on immune system cells related to cell growth, proliferation, apoptosis and SLE pathophysiology. Vitamin D suppresses B and T cell proliferation, differentiation and immunoglobulin secretion. Thus, vitamin D inhibits the secretion of inflammatory cytokines such as IL-17 while increasing the anti-inflammatory cytokine levels such as IL-10 [5,6]. In our study, in the light of the above information, we intended to measure IL-10, IL-17 and IL-23 cytokines and vitamin D levels in SLE patients, which we think may be effective in the pathogenesis of the disease, and to investigate their possible relationship with clinical and laboratory data.

Materials and Methods

Patient Selection

Our study was validated by the Adnan Menderes University Medical Faculty, Ethics Committee on 12.09.2013 with the number 56989545/050.04-201. Forty patients diagnosed with SLE who were followed in Adnan Menderes University Medical Faculty Internal Medicine Rheumatology Clinic and 20 healthy controls similar in sex and age to the SLE cases. Those included in the control group had no medical history and were not using any medication. SLE patients were selected according to the American Rheumatology Association criteria [7]. SLE disease activation scores (SLEDAI) were calculated by determining the clinical findings and organ involvement of the patients in terms of SLE [8]. All SLE patients participating in our study had received or continued treatments such as hydroxychloroquine, steroid and/or cyclophosphamide. Those who used antiepileptic drugs or anticoagulants and those who received vitamin D replacement within 6 months in the control and patient groups were not included in the study.

Laboratory Analysis

In order to evaluate the kidney involvement due to SLE, complete urine analysis and 24-hour urine analysis of the patients were taken into consideration. After excluding stones, infections, and other causes, patients with 5 or more erythrocytes at high magnification were considered hematuric. Patients with proteinuria more than 500 mg a day and patients whose protein excretion increased by >500 mg according to their previous examinations were considered proteinuric. After the infection was excluded, seeing 5 or more white blood cells at high magnification under the microscope was considered as pyuria. Serum complement (C3, C4) levels of the patients were measured by nephelometric method (reference range C3: 85-200 mg / dL, C4: 20-50 mg / dL), anti-double stranded DNA (anti-ds DNA) levels were measured by ELISA technique. In the whole blood test performed in terms of hematological findings, platelets were evaluated as <100.000/mm3 thrombocytopenia, and <4000/mm3 white blood cell leukopenia after drug-related causes were excluded. Direct and indirect Coombs tests were performed for immune hemolytic anemia in patients with anemia. After 5 cc of blood taken from the cases was centrifuged at 3000 rpm for 10 minutes, their serum samples were separated to measure IL-10, IL-17 and IL-23. Diasource ELISA Human IL-10 (Belgium) kit was used for IL-10 measurement. The kit was studied with the sandwich ELISA method, which gave quantitative results. 100 µl of serum samples and standart were put in to the wells and it was incubated for two hours at 21 degrees Celcius. Then, 100 µl serum sample and 50 µl anti-IL-10-HRP Conjugate were added to the wells. It was incubated at room temperature with gentle stirring for two hours and the solutions were washed. Finally,

50 µl of the reaction stopping solution was added to the wells, readings were made at 450 and 490 nm in the spectrophotometer. The results read here were multiplied by 2, taking into account the dilution factor. The Ebioscience ELISA Human IL-17 (United States) kit was used for IL-17 measurement. The kit was studied with the sandwich ELISA method, which gave quantitative results. A mixture of 100 µl serum and standart were incubated for 2.5 hours at 21 degrees Celcius. Then 100 µl biotinylated antibodies were put into each well. It was incubated at room temperature with gentle stirring and shaking for one hour. After incubation, the solutions were washed and 100 µl streptavidin solution was added and incubated at 21 degrees Celcius for 45 minutes. After the solutions were washed properly, 100 µl of the composition named TMB substrate solution was put into wells and incubated for 30 minutes. Finally, 50 µl of reaction stopping solution was put into the wells and read at 450 nm in the spectrophotometer. The results read here were multiplied by 2, taking into account the dilution factor. The Ebioscience ELISA Human IL-23 (United States) kit was used for IL-23 measurement. The kit was studied with the sandwich ELISA method, which gave quantitative results. Similarly, after removing all serum samples and standards and washing appropriately, 100 µl Biotin Conjugate, 100 µl Avidin HRP and 100 µl TMB Substrate solutions were added respectively. After 15 minutes of incubation at room temperature, the color change of each plate was evaluated and 100 µl of the reaction stopping solution was added and the absorbances were read at 450 nm in the spectrophotometer. The results read here were multiplied by 2, taking into account the dilution factor. 25 (OH) vitamin D was serologically studied by HPLC (high-performance liquid chromatography) method. Evaluation for 25 (OH) vitamin D level; <10 ng / ml severe deficiency, <20 ng / ml deficiency, 20-30 ng / ml insufficiency, 32-100 ng / ml is considered sufficient.

Statistical Analysis

Medcalc 9 (Acacialaan 22, B-8400 Ostend, Belgium) and Statistical Package for the Social Sciences (SPSS) 22 programs were used to analyze. The compliance of the data for normal distribution was examined with Kolmogorov-Smirnov test. To compare two independent groups, Independent-Samples T test was used. Mann-Whitney U test was used with Monte Carlo simulation technique. Quantitative data are expressed as mean ± SD and median Range (max-min) values in the tables. Categorical data are expressed as n (number) and percentages (%). The data were analyzed at 95% confidence interval, and cases where the p value was less than 0.05 were considered as statistically significant.

Results

In our study, 40 patients diagnosed with SLE whose treatments are ongoing and 20 healthy controls were evaluated retrospectively. Thirty eight (95%) of the SLE patients were female and 2 (5%) were male. The control group was 6 (30%) males and 14 (70%) females. The mean age of the patient group was 35.5±13.4 years; mean duration of disease was found to be 6.1 ±5.9 years. The mean age of the control group was 36.1 ±14.7 years. The clinical manifestations of SLE patients are summarized in Table 1. Using these data, the mean SLEDAI score of all patients was calculated as 6.95. The SLEDAI score of 13 (32.5%) patients was found to be between 0-3, 17 (42.5%) patients between 4-10, and 10 (25%) patients as ≥ 11. Serum IL-10 levels in the control and patient groups were compared, no statistically significant difference was found (p=0.333). Similarly, IL-17 levels of the control and patient groups were compared. It was not statistically significant different (p=0.99)

Table 1. Demographic, laboratory and clinical characteristics of the patients and the control group

	SLE patients n:40 (%)	**Control n:20 (%)**
Mean Age	35.5 ±13.4	36.1 ±14.7
Gender (Female)	38 (95%)	14 (70%)
Mean Duration of Disease (years)	6.1 ±5.9	
Skin Lesion	25 (62.5%)	
Kidney Involvement	26 (65%)	
Joint Involvement	4 (10%)	
Hematological Involvement	6 (15%)	
Low C3	17 (42.5%)	
Low C4	25 (62.5%)	
SLEDAI Score (mean)	6.95	

Table 2. IL-10 and IL-17 levels of SLE and control group

	Control		SLE		P value
	Median (Max-Min)	Mean	Median (Max-min)	Mean	
IL-10 (pg/ml)	8.33 (181.19-8.33)	65.94	55.44 (547.63-8.07)	99.72	0.333
IL-17 (pg/ml)	11 (22-5)	12.14	12.15 (177.26-2.74)	17.23	0.990

Table 3. IL-23 levels of SLE and control group

	n	IL-23 Mean (pg/ml) ±SD	P value
Control	20	345.53 ±191.86	p<0.001
Patients	40	921.79 ±607.23	

Table 4. 25 (OH) vitamin D levels in SLE patients and control group

	n	25 (OH) vitamin D Mean (ng/ml) ±SD	P value
Control	20	22.63 ±8.1	p=0.003
Patient	40	15.87 ±6.47	

(Table 2). When we compare serum IL-23 levels of the control and patients, IL-23 levels of the SLE patients were higher than the control group and statistically significant (p<0.001) (Table 3). The relationship between IL-23 levels and SLEDAI scores and disease duration of the patient group was evaluated. There was not relationship between disease duration or SLEDAI score and IL-23 (p=0.476). While the mean IL-23 levels of 26 patients with active kidney pathology were 966.82 pg/ml, the mean IL-23 levels of 14 patients without active kidney pathology were 896.6 pg/ml. Although the mean IL-23 levels of patients with active kidney pathology were found to be higher than those without, there was not statistically significant difference between them (p=0.734). When the 25 (OH) vitamin D levels of the SLE and control groups were compared, the vitamin D levels of the patient group (mean 15.87 ng/ml) were lower than the control group (mean 22.63 ng/ml) and were statistically significant (p=0.003). Vitamin D was sufficient 4 (20%) of the control group and 9 (45%) of the control group had vitamin D insufficiency, 6 (30%) had vitamin D deficiency, and 1 (5%) had severe vitamin D deficiency. Vitamin D was sufficient in 1 (2.5%) of the patient group and 7 (17.5%) had vitamin D insufficiency, 25 (62.5%) had vitamin D deficiency and 7 (17.5%) had severe vitamin D deficiency (Table 4). The relationship between vitamin D levels and SLEDAI score, disease duration, renal involvement, steroid and hydroxychloroquine use was evaluated in the patient group. It was observed that the longer the disease duration, the lower the vitamin D levels and the results were statistically significant (p=0.020) (Figure 1). There was not significant relationship between the SLEDAI score, renal involvement, hydroxychloroquine or steroid use and vitamin D levels (p=0.247, p=0.634, p=0.927 and p=0.562 respectively). Serum IL-10, IL-17 and IL-23 and vitamin D level were compared. While no

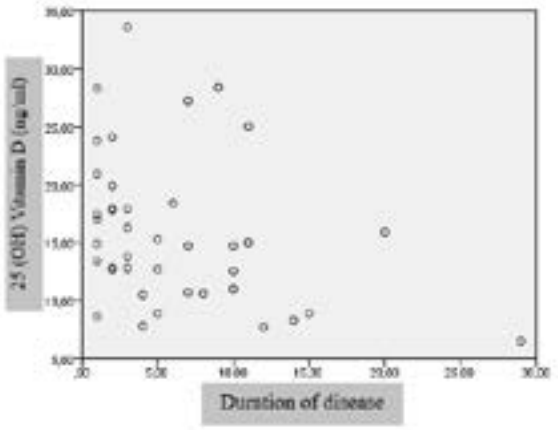

Figure 1. Negative correlation between vitamin D levels and disease duration.

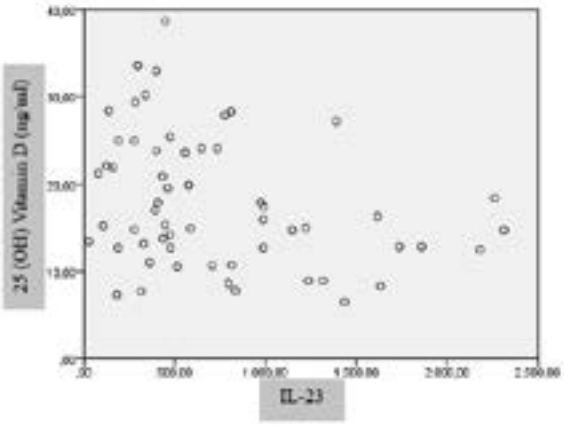

Figure 2. Negative correlation between IL-23 and vitamin D levels.

significant relationship was found between IL-10 and IL-17 levels and vitamin D, a statistically significant relationship was found between vitamin D and IL-23 (p=0.019) (Figure 2).

Discussion

In our study, vitamin D levels and serum IL-10, IL-17, IL-23 levels were measured in control and patient groups similar in age and gender. There was not significant difference between the patient and control groups in terms of IL-10 and IL-17 levels, so the relationship between serum IL-10 and IL-17 levels couldn't be evaluated with clinical and laboratory data of the patients. Our results have been found different from some previous studies on this subject. IL-10 levels were investigated in the study of Waszczykowska et al. (1999), which consisted of 63 SLE patients and 16 healthy control groups. IL-10 levels increased three times in SLE patients compared to the healthy controls and it was correlated with SLEDAI score ($p<0.001$) [9]. In our study, 39 patients (97.5%) received any immunosuppressive treatment, whereas 35 patients (55.5%) received treatment in this study. In addition, while 25% of the patients were active in our study, 50.8 % of them were active in this study. The different outcomes between two studies might be related to differences such as treatment rates and disease activity among the patients included in the study. Wong et al. (2008) compared IL-17 and IL-23 in 80 SLE patients and 40 control groups. IL-17 and IL-23 levels were higher in SLE patients compared to controls ($p<0.05$) [10]. However, in our study, no significant difference was observed between the groups in terms of IL-17, while IL-23 levels were found significantly higher in SLE patients. In the study of Wong et al. (2008), while IL-17 level correlated with disease activity in the SLE group without renal involvement, no significant difference was observed between the groups in terms of disease activity in IL-23. In our study, there was not significant relationship between IL-23 and disease activity, duration or renal involvement. This data we have obtained is similar to the work of Wong et al. Considering the differences between the two clinical studies, the mean SLEDAI score of the patients was 8, while it was 6.95 in our study. In this study, 50% of the patients had kidney involvement, while in our study, kidney involvement was 35%. While the mean disease duration was 6.1 years in our study, it was 12.4 years in this study. Many factors such as patients' mean SLEDAI scores, renal involvement, duration of disease may be the reason for the difference in results in terms of IL-17. SLE is a complex disease that causes autoimmune inflammation and can cause many events in the immune system. Therefore, when the immune, genetic and environmental mechanisms are fully understood, specific inflammatory pathways that mediate the disease can be targeted. Recent studies in the literature suggest that IL-23 may play a role in the pathogenesis of SLE, correlate with disease activity and be a predictor for response to immunosuppressive therapy. In the study of Mok et al. (2010) serum IL-23 was found to be elevated in active SLE patients who presented with cutaneous manifestations and serositis, further supporting a role of IL-23/Th17 in the pathogenesis of SLE [11]. It is important to note that not all manifestations of SLE are associated with increased IL-23 levels, suggesting the variability in the mechanisms of manifestations in SLE [12]. In another study by Dedong et al. (2019), it was mentioned that IL-23 can be used as a predictor in response to immunosuppressive therapy in patients with active lupus nephritis [13]. Specific blocking of the IL-23 immune pathway can be an effective and safe treatment option in the treatment of many autoimmune diseases, including SLE. In a multi-center study investigating the efficacy and safety of IL-12 and IL-23 inhibitor ustekinumab in SLE patients, the primary end point was SRI4 (SLEDAI-2K responder index) response. In the study, ustekinumab provided significant improvement compared to placebo [14]. In the study of Shahin et al. (2017), 25 (OH) vitamin D, IL-17 and IL-23 levels were compared in 57 SLE patients and 42 control groups [15]. Vitamin D levels were significantly lower in SLE patients ($p=0.001$). A negative correlation was found between the vitamin D level and IL-17 and IL-23 ($p<0.05$). These data are similar to the results of our study. Vitamin D is involved in bone metabolism and has immune regulatory functions as well as a nutrient source for many tissues and organs. When the vitamin D levels were examined, vitamin D levels were lower in SLE patients, and a negative correlation was found between the disease duration and IL-23. In our study, although 85% of patients in the SLE group used corticosteroids, no relationship was found between vitamin D and corticosteroid use. However, the regularity of corticosteroid use and the inability to measure cumulative doses should be considered. Hydroxychloroquine can lead to vitamin D deficiency by inhibiting 1α hydroxylation of 25(OH) D. However, in our study, no significant relationship was found between vitamin D levels and hydroxychloroquine use. Due to the high prevalence of vitamin D deficiency in healthy individuals, it may be recommended to measure vitamin D at the time of SLE diagnosis. Serum cytokine concentrations are affected by many factors

such as production, tissue or cell storage, degradation and elimination [16].

Conclusion

In conclusion, we evaluated vitamin D and cytokine levels in SLE patients receiving treatment. However, if the patient groups in whom treatment was not initiated were examined, we would have obtained different results. Since SLE is a disease with low prevalence, the number of cases included in our study is one of the limitations of our study. Future studies will be needed whether IL-23 levels might be a biomarker of lupus as well as predictor of response to biologics targeting the IL-23 pathway.

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Conflict of interest
The authors have no conflicts of interest declared.

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