Research Article

The Cytokine Genetic Polymorphism of Interleukin-10 and Interleukin-12 in Systemic Lupus Erythematosus Disease

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Abstract

Background: Systemic lupus erythematosus is a systemic autoimmune disease or autoimmune connective tissue disease in which the body's immune system incorrectly attacks healthy tissue. Cytokine imbalance has been demonstrated in systemic lupus erythematosus patients.

Objective: The aim of this study was to clarify the cytokine genetic polymorphisms of interleukin-10, interlukin-12, and systemic lupus erythematosus.

Patients and Methods: This study was conducted in Medical City Hospital through the period from June 2013 until the end of October 2014. Fourty six female patients with systemic lupus erythematous were enrolled in this study; their age range was (18-45) years and they were compared with 44 healthy controls. About 5 ml of blood sample was obtained from every systemic lupus erythematosus patient and control. The separated serum was used for measurements of interleukin-10, interleukin-12, and cytokine genetic polymorphism. **Results:** Interleukin-10 and interlukin-12 showed a significant increased level in patients as compared to the controls ($P \leq 0.001$). Genetic polymorphisms of tow cytokine genes (interleukin-10 and interleukin-12) at 4 positions (interleukin 10_{-1082}, interleukin 10_{-819}, interleukin 10_{-592} and interleukin 12_{-1188}) were determined in patients and the control.

Conclusions: It can be concluded that serum interleukin-10 and interleukin-12 was increased in patients as compared to the control group, so the declining its concentration would help prevention of systemic lupus erythematosus disease.

Key words: Systemic lupus erythematosus, interleukin-10, interleukin-12, cytokine genetic polymorphism.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease that is connected with involvement of various organs and mostly affects women of childbearing age. The prevalence of SLE in the general population is approximately one in 2000, with a nine to one female gender bias and a two to four fold greater prevalence in non-Caucasian compared with Caucasian populations [1]. The cellular and molecular mechanisms governing inflammation in SLE remain uncertain; however, the etiology and pathogenesis of SLE involve genetic, epigenetic, immunological and environmental factors, and additionally it is a heterogeneous disease with highly variable staging[2]. The one feature common to virtually all SLE patients is the existence of antinuclear autoantibody, which is considered a hallmark of the disease[3]. More than 100 probable genetic risk factors for SLE have been well-known through case studies, association analyses of

multiplex families, and candidate gene case-control studies[4,5].

Systemic lupus erythematosus has an important genetic component, as familial aggregation studies have shown that siblings of SLE patients have a greater comparative risk for the disease compared with the population as a complete [6]. There is, for example, a 10-fold increase in the concordance rate of SLE in monozygotic twins compared with dizygotic twins [7]. In addition to heredity, cytokine imbalance has been demonstrated in SLE patients. Therefore, hereditary polymorphisms in cytokine genes, which manipulate gene expression and cytokine production, may have an important impact on SLE susceptibility and severity. In this regard, it has been found that genotype-22 of interleukin 12 (IL12)Bpro was predominantly combined with genotype-AA of single nucleotide polymorphisms (SNP) in 3'UTR among SLE patients, and this combination was suggested to raise the risk of SLE[8].

Numerous cytokines have been concerned in the disease activity or organ interest in SLE. Among these, IL-6, Interferon (IFN), B-lymphocyte stimulator (BlyS), IL-10, IL-17 is thought to play a vital role in the creation of the feature milieu in SLE, which promotes B-cell survival and autoantibody production. On the other hand, also cytokines like IL-10, IL1, TNF, IFN, are essential in progress of the autoimmune injury in renal and central nervous system, the most often observed causes of death in patients with SLE [9]. The IFN-γ is known to stimulate synthesis of IL-12 by antigen-presenting cell (APC) [10].

The aim of this study was to clarify the cytokine genetic polymorphisms of IL-10, IL-12, and SLE.

PATIENTS AND METHODS

This study was conducted in the Medical City Hospital through the period from June 2013 until the end of October 2013. Fourty six patients were enrolled in this study; their age range was (18-45) years and compared with 44 healthy subjects as a control group. They studied for their third and fourth complement (C3 and C4) Components anti-nuclear antibodies (ANAs) and anti-double strand DNA antibodies (dsDNA). Also IL10 and IL12 were measured in those patients and compared with the control group. From each participating subject five milliliters of blood was aspirated from a suitable vein. The blood sample was immediately divided into two aliquots. The first aliquot (2 ml) was transferred to a sterile EDTA tube and stored at -20ºC until assayed for cytokine polymorphism genotyping .The second aliquot (3 ml) was transferred to a plain tube, and then it was left to clot at room temperature (20-25°C) for 15 minutes, centrifuged at 1000 rpm for 10 minutes to separate the serum, which was divided into aliquots (0.25 ml) in tightly closed Eppendrof tubes, and by then they were stored at -20ºC until assayed for the serum level of cytokines.

Interleukin 10 and Interleukin 12:

The serum cytokine concentrations of SLE patients and control subjects were measured by enzyme-linked immunosorbent assay (ELISA) using OptEIA reagent kits (Pharmingen, BD Biosciences, San Diego, CA) according to the manufacturer's instructions.

DNA Extraction:

Genomic DNA was extracted from ethylene diamine tetra acetic acid (EDTA) blood using the Wizard® Genomic DNA Purification Kit (USA), which is designed for manual separation and purification of genomic DNA from fresh, frozen or old human blood with ordinary anticoagulants (EDTA and citrate).

Assessment of DNA Yield:

The DNA yield was spectrophotometrically assessed using Cecil E1021 spectrophotometer (England), in which the sample was read at two optical densities that were 260 and 280 nm. The second reading was divided by the first reading, and if the outcome was 1.8-2.0, the sample was considered as free of contamination and having a sufficient amount of DNA for a further analysis. The DNA concentration was calculated using the following formula as given by Sambrook et al. (1989).

DNA Concentration (µg /µl) = OD at 260 nm× Dilution Factor ×50µg/ml

Cytokine Genetic Polymorphism:

Each of the cytokine CTS-PCR-SSP tray kit contains PCR primer mixes pre pipetted and dried in thin-walled, plastic, green 96-well PCR trays for cytokine genotyping of two individuals (48 PCR primer mixes for each individual). Each kit provides 10 trays for 20 typings in total. Cytokine genotyping commercially available PCR-SSP kit (Heidelberg kit, cytokine genotyping tray, Invitrogen, GmbH, Karlsruhe, Germany) was used. Fourteen cytokine genes with 22 SNP were typed: IL-1alpha -889, IL-1beta -511, IL-1beta +3962, IL-1R psti1970, IL-1RA mspa11100, IL-4Ralpha +1902, IL-12 -1188, IFNgamma utr5644, TGF-beta1 cdn10,TGF-beta1 cdn25, TNF-alpha -308, TNF-alpha -238,IL-2 -330, IL-2 +166, IL-4 -1098, IL-4 -590, IL-4 -33,IL-6 -174, IL-6 565, IL-10 -1082, IL-10 -819, and IL-10-592. Briefly, PCR-SSP typing Heidelberg kit consists of 48 PCR primer mixes aliquoted in 96-well PCR trays (two typing per tray). Master mix, which was supplied along with the reagents and consisted of

$MgCl_2$, buffer, dNTP's, and glycerol was mixed with 1.2-3.0 µg DNA and 20 U Taq polymerase and dispensed in 48 wells. Agarose gel electrophoresis on a 2% gel revealed a positive or negative signal for particular amplification in each well. Consequently, the results were analyzed according to the explanation design provided by the kit.

Statistical Analysis:

Statistical methods included two main analyses; cytokine genetic polymorphism and disease association and assessment of significance between means of cytokine serum levels. The analyses for CGP and disease association were adopted from (Kaur and Mehra).

The association between a marker and a disease was expressed in terms of relative risk (RR), etiological fraction (EF) and preventive fraction (PF). The RR value can range from less than one (negative association) to more than one (positive association). If the association was positive, the EF was calculated, while if it was negative, the PF was calculated. The significance of such association (positive or negative) was assessed by Fisher's exact probability (P). Such assessment is more preferred, because it is not affected by small numbers (less than 5).

The serum level of cytokines was analyzed by the computer programmer SPSS (Statistical Package for Social Sciences) version 13. Their data were offered in terms of means ± standard errors (S.E.), and the differences between the means were assessed by ANOVA, LSD and Duncan's tests. The difference was considered significant when the probability (P) value was ≤ 0.05.

RESULTS

Serum Level of Cytokines:

Levels of two cytokines (IL-10 and IL-12) were assessed in sera of SLE patients and controls. Levels of IL-10 showed a highly significant increase in SLE patients when compared with the controls, ($P \leq 0.001$). Also IL-12 showed a highly significant increase in SLE patients compared to the controls, ($P \leq 0.05$).

Cytokine Gene Polymorphisms:

Genetic polymorphisms of tow cytokine genes (IL10, and IL12) at 4 positions ($IL10_{-1082}$, $IL10_{-819}$, $IL10_{-592}$, and $IL12_{-1188}$) were determined in SLE patients and the controls group. They were genotyped by polymerase chain reaction with sequence specific primers (SSP-PCR) method.

Interleukin 10_{-1082} Genotypes:

Two different homozygous $IL10_{-1082}$ genotypes (AA in SLE patients and GG in the controls) were associated with the highest serum level of IL-10 (37.1 ± 2.5 and 38.8 ± 5.7 pg/ml, respectively), but the difference was only significant in the controls as compared to the other two genotypes (GA and AA), in which a reduced level was observed (30.7 ± 1.9 and 29.3 ± 1.0 pg/ml, respectively). In SLE patients, although a decreased serum level of IL-10 in GG and GA genotypes as compared to AA genotypes, the difference was not significant, figure 2.

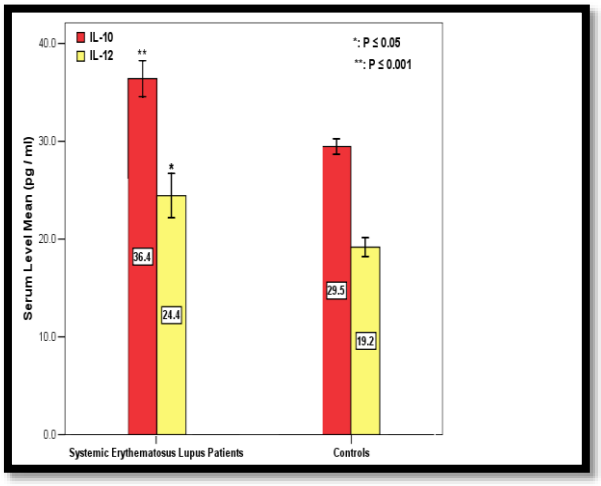

Figure 1: Serum levels of IL-10 and IL-12 in systemic lupus erythematosus patients and the controls

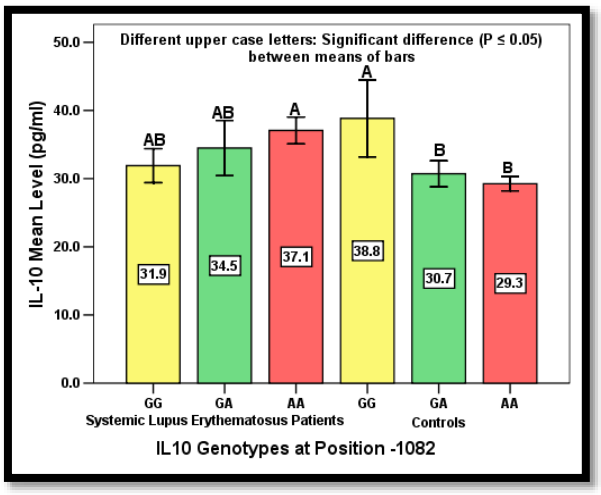

Figure 2: Serum level of IL-10 in $IL10_{-1082}$ genotypes of systemic lupus erythematosus patients and the controls

Interleukin 10_{-819} Genotypes:

As shown in figure 3, control subjects recorded a similar serum level of IL-10 (30.5 ± 1.2, 29.6 ± 1.1 and 26.7 ± 2.3 pg/ml, respectively) in the three genotypes (CC, CT and TT) of IL-10 gene at position -819, while in SLE patients, a variation was observed. The genotype TT recorded the highest level (42.1 ± 6.4 pg/ml) followed by CT and CC genotypes (36.4 ± 2.1 and 32.6

± 1.9 pg/ml, respectively), but a significant difference was observed between TT and CC genotypes.

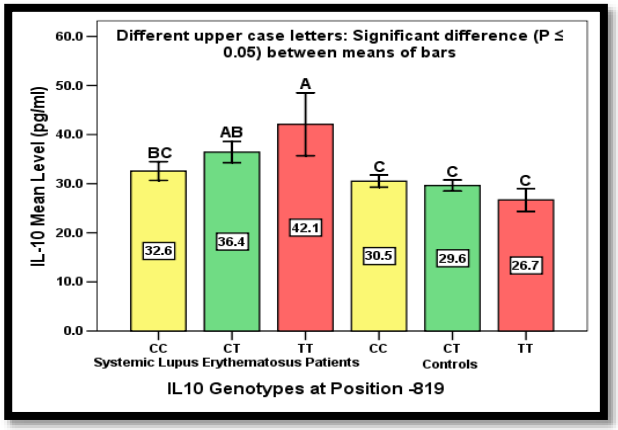

Figure 3: Serum level of IL-10 in IL10$_{-819}$ genotypes of systemic lupus erythematosus patients and the controls

Interleukin 10$_{-592}$ Genotypes:

Among SLE patients, the IL10$_{-592}$ AA genotype recorded the highest serum level of IL-10 (45.9 ± 8.9 pg/ml), which was significantly different compared to the other two genotypes (CC: 34.3 ± 2.4 and CA: 34.9 ± 1.7 pg/ml). In contrast, no such differences were observed between CC, CA and AA genotypes in the controls (29.8 ± 1.4, 29.3 ± 1.1 and 29.4 ± 2.1 pg/ml, respectively), figure 4.

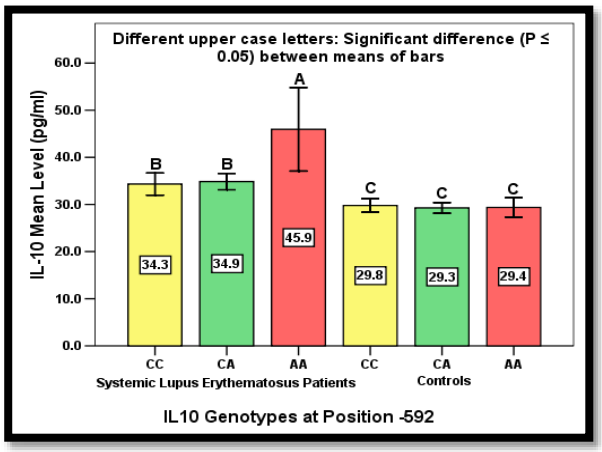

Figure 4: Serum level of IL-10 in IL10$_{-592}$ genotypes of systemic lupus erythematosus patients and the controls

Interleukin 12$_{-1188}$ Genotypes:

A serum level of IL-12 (35.7 ± 6.5 pg/ml) was observed in SLE patients with the genotype AA of IL12 gene at position -1188. Such level was significantly higher than the recorded level in AC and CC genotypes (20.5 ± 2.8 and 23.8 ± 3.2 pg/ml, respectively). In controls, the AC genotype was observed with the highest level of IL-12 (22.1 ± 1.9 pg/ml), and the difference was significant compared to AA and CC genotypes (16.2 ± 2.3 and 18.5 ± 0.9 pg/ml, respectively), figure 5.

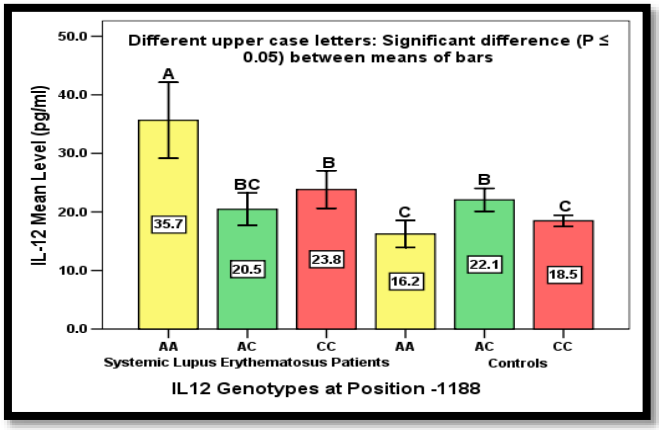

Figure 5: Serum level of IL-12 in IL12$_{-1188}$ genotypes of systemic lupus erythematosus patients and the controls

DISCUSSION

This search has focused in the new insights about the role of cytokine in the pathogenesis of SLE. The imbalance in the levels of cytokines and their receptors establish in SLE is clearly crucial to the progress of the pathology of the disease.

Interleukin-10 is a key immunoregulatory cytokine that can be produced by almost all leukocytes, including innate immune cells such as monocytes, macrophages, DCs, mast cells, natural killer cells, eosinophils, and neutrophils, and adaptive immune cells. The most important targets of IL-10 on immune cells are antigen presenting cells and lymphocytes[11].

On the one hand, IL-10 repressed the antigen-presenting capability of monocytes and macrophages by downregulating cell surface levels of MHC classII, co-stimulatory molecules such as CD86 and adhesion molecules such as CD58. Interleukin-10 also repressed the function of DCs by downregulating the production of IL-12 and expressions of MHC class II and co-stimulatory molecules[12]. In addition, the stimulatory effect of IL-12 on TH1 development is antagonized by IL-4, a cytokine which promotes TH2 cell development. Therefore, IL-12 plays an essential role in cell-mediated inflammation and also contributes to the instruction of immunoglobulin production. Interleukin-10, a B-cell stimulatory cytokine is identified to inhibit type 1 cytokine response and has a significant role in the immunopathogenesis of SLE. Spontaneous and mitogen induced IL-10 levels have been reported to be elevated in SLE patients [13].

The levels of IL-10 were extraordinarily elevated in SLE patients as compared to the healthy controls. Additionally. The plasma level of IL-12 in

active SLE patients was found to be elevated than in the controls as reported as a result of Wong et al, 2000 [14].

In the current study, levels of serum IL-12 were elevated and positive relationship with systemic lupus erythematosus patients, which is concurrence with the study of Tucci et al, 2008 [15]. This inconsistency in the result may be due to diverse demonstration of SLE disease.

Conclusions:

It can be concluded that serum IL-10 and IL-12 was increased in patients as compared to the controls group. Interactions between the cytokine milieus are complex and the attenuation of one cytokine would require to be approached with concern, considering effects on the cytokine arrangement as a whole. There are still many facets of immunopathology of SLE elicited by cytokines to be elucidated.

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