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IL10 in Lupus Nephritis: Detection and relationship with disease activity

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Abstract

Introduction: Glomerulonephritis is a major determinant of the course and prognosis of systemic lupus erythematosus (SLE) and is evident in 40%–85% of patients. IL10, a cytokine produced by monocytes and-to a lesser extent-lymphocytes, has pleiotropic effects in immune regulation and inflammation. It enhances B cell survival, proliferation, differentiation, and antibody production; these effects play a role in autoimmune diseases. Among identified polymorphisms in the IL10 promoter, three linked single nucleotide polymorphisms (SNPs) of -1082 G/A, 819 T/C, and -592 A/C have been shown to influence the IL10 gene expression. Compared with the -592 C allele, the 592 A is associated with lower IL10 production in vitro. The objectives of this study were to investigate the -592 A/C polymorphism in patients with and without lupus nephritis and to assess its influence on IL10 secretion in vivo and its role in pathogenesis and clinicopathological characteristics of lupus nephritis.

Methods: This case control study was conducted on 40 SLE patients recruited for the study from those attending the nephrology department of the Theodor Bilharz Research Institute (outpatient clinic and inpatient ward) in 2013. Patients were divided into two groups, group I (SLE patients without evidence of nephritis) and group II (SLE patients with lupus nephritis). Data were analyzed using SPSS (version 12), a t-test, Chi square, ANOVA, and the Pearson product–moment correlation coefficient.

Results: Our study found an increase in IL10 serum in lupus nephritis patients compared to those without renal involvement (without statistical significance). No significant differences emerged in the level of IL10 serum among different pathological classes.

Conclusion: The IL10 gene (-592 A/C) polymorphism, though not associated with lupus nephritis's susceptibility in the present study, does play a role.

Keywords: systemic lupus erythematosus, IL10, lupus nephritis

1. Introduction

Glomerulonephritis is a major determinant of the course and prognosis of SLE and is evident in 40%–85% of patients (1). Raised titers of anti-DNA and hypocomplementemia have been reported to be associated with activity of the disease (2). However, their non-specificity led to the search for other antibodies that might contribute to nephritis and help diagnose renal flare (3). IL10, a cytokine produced by monocytes and to a lesser extent lymphocytes, has pleiotropic effects in immune regulation and inflammation (4). It enhances B cell survival, proliferation, differentiation, and antibody production, and these effects play a role in autoimmune diseases (5). A 40-fold increase in risk for developing SLE was identified in individuals with particular alleles of both IL10 and bcl2 genes (6), and it has been considered that polymorphisms of IL10 contribute—at least in part—to the genetics involved in SLE. Among identified polymorphisms in the IL10 promoter, three linked single nucleotid polymorphisms (SNPs) of -1082 G/A, 819 T/C, and -592 A/C have been shown to influence the IL10 gene expression (7). Compared with the -592 C allele, the 592 A is associated with lower IL10 production in vitro (8). This study investigated the -592 A/C polymorphism in patients with and without lupus nephritis and assessed its

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influence on IL10 secretion in vivo and its role in pathogenesis and clinicopathological characteristics of lupus nephritis.

2. Material and Methods

Forty SLE patients (34 females and 6 males) were recruited in the study from those attending the nephrology department of the Theodor Bilharz Research Institute (outpatient clinic and inpatient ward) in 2013. Written consent was acquired from all participants after explaining the details, benefits, and risks to them. Patients were divided into two groups according to clinical presentations and laboratory investigations. Group I comprised 15 SLE patients (13 females and 2 males) without evidence of nephritis. Group II comprised 25 SLE patients (21 females and 4 males) diagnosed with lupus nephritis according to American College of Rheumatology (ACR) criteria (i.e., proteinuria > 500 mg/day and/or red cell casts). The diagnosis of renal involvement was confirmed by renal biopsy.

The biopsies were classified according to the International Society of Nephrology/Renal Pathology Society. Classes III and IV are considered more active while classes I, II, and IV are considered less active. Exclusion criteria were: SLE patients with proteinuria other than lupus nephritis, such as pregnancy and fever, and/or patients with renal impairment due to any other cause than lupus nephritis, such as diabetic nephropathy. In addition, patients with HCV, HBV, and other connective tissue diseases other than SLE were excluded from the study.

All patients were subjected to the following: (1) complete history and physical examination; (2) routine laboratory investigations, including complete blood count, erythrocyte sedimentation rate, serum urea, serum creatinine, C-reactive protein, creatinine clearance, urine analysis, and 24-hour protein excretion; 3) immunological profiles, including antinuclear antibody (ANA) by indirect immune-fluorescence principle, Anti-ds DNA by solid phase immunoassays, and complement level (C3 and C4) by using BN ProSpee nephelometery; and 4) serum level of IL10 by indirect solid phase enzyme immunoassay (ELISA).

Results were expressed as means ± standard deviation (SD) of the means or number (%). Different parameters in the two studied groups were compared using an unpaired t-test. Parameters in different pathological classes were compared using ANOVA. Categorical data were compared using a Chi square test. The correlation between different parameters in the cases group was defined using the Pearson product–moment correlation coefficient. The data were considered significant if the p-value was equal to or less than 0.05 and highly significant if the p-value < 0.01. Statistical analyses were performed with the aid of the SPSS computer program (version 12, Windows).

3. Results

Forty systemic lupus erythromatosis patients from the nephrology department of TBRI were included in the present study (34 females and 6 males). They were classified into two groups: 15 without nephritis and 25 with evidence of lupus nephritis (8 patients has class III, 11 patients has class IV, and 6 patients has class V nephritis). All patients were analyzed for the presence of the 592 A/C polymorphism. The demographic, clinical and laboratory data are summarized in Tables 1, 2, and 3. As shown in Table 3, a significant difference existed between group I and II in terms of creatinine, BUN, urea, creatinine clearance, protein in urine/24 hours, C3, C4, and anti-dsDNA. In addition, the mean values of IL10 in the two groups studied were 5.11 ± 7.58 and 14.66 ± 39.14, and no statistically significant difference emerged between group I and II in terms of IL10 ($p = 0.358$). Table 4 shows the correlation analysis between IL10 and the different parameters studied in group II according to the Pearson product–moment correlation coefficient. Tables 5 and 6 present the genotype frequency and alleles' frequency in the two groups studied. Table 7 shows the comparison between mean values of IL10, Creatinine clearance and 24-hour protein measured before and after treatment in group II. Finally, Table 8 summarizes the results of the comparison between mean values of IL10 measured before and after treatment in the cases group (group I).

Table 1. Comparison of demographic data in two groups studied

Demographic variables	Group *I* (n=15)	Group II (n=25)	p-value
Age	29.4 ± 7.02	27.68 ± 7.03	0.458
Gender (F/M)	13/2 (86.7%/13.3%)	21/4 (84%/16%)	0.819
Duration of disease (yrs.)	1.55 ± 1.13	2.20 ± 1.59	0.168

Data are expressed as mean ± SD or a number (%)

Table 2. Comparison of clinical manifestations in two groups studied

Variables		Group *I* (n=15)	Group *II* (n=25)	p-value
Dermatological manifestations	Malar rash	15 (100%)	12 (48%)	0.003
	Discoid rash	0 (0%)	1 (4%)	0.433
	Photosensitivity	11 (73.3%)	19 (76%)	0.850
	Hair fall	14 (93.3%)	15 (60%)	0.022
	Oral ulcer	8 (53.3%)	15 (60%)	0.680
Articular manifestations	Arthralgia	13 (86.7%)	16 (64%)	0.120
	Fever/malaise	8 (53.3%)	7 (28%)	0.109
	Seizure	2 (13.3%)	2 (8%)	0.586
	Psychosis	0 (0%)	0 (0%)	---
	Serositis (pleural effusion)	0 (0%)	7 (17.5%)	0.163

Data are expressed as a number (%)

Table 3. Comparison of laboratory manifestations in two groups studied

Variables		Group *I* (n=15)	Group II (n=25)	p-value
Complete blood count (CBC)	Hb (g/dL)	10.61 ± 1.02	10.31 ± 1.62	0.516
	RBCs ($\times 10^6/\mu L$)	3.87 ± 0.53	4.06 ± 0.39	0.203
	WBCs ($10^9/L$)	7.09 ± 3.93	6.21 ± 2.09	0.361
	Platelet count ($10^9/L$)	243.87 ± 45.81	206.96 ± 70.44	0.079
S. creatinine		0.58 ± 0.12	1.76 ± 1.63	0.001
BUN		11.77 ± 2.94	39.88 ± 38.80	0.001
Urea		27.33 ± 7.11	67.32 ± 68.99	0.008
Protein 24 hrs.		121.33 ± 23.29	2587.00 ± 2439.50	0.001
Creatinine clearance		107.07 ± 7.46	67.80 ± 28.66	0.001
CRP		6.63 ± 5.31	6.92 ± 6.06	0.876
ESR		67.27 ± 39.00	88.08 ± 27.80	0.056
C3		1.13 ± 0.17	0.63 ± 0.49	0.001
C4		0.74 ± 0.61	0.26 ± 0.42	0.012
Anti-dsDNA		59.19 ± 23.96	169.71 ± 162.47	0.003

Data are expressed as mean ± SD

Table 4. Correlation between IL10 and different parameters studied in group II

Variables	Pearson product–moment correlation coefficient	Sig. (2-tailed)
Urea	-0.179	0.392
BUN	-0.159	0.448
Serum creatinine	-0.169	0.421
ESR	-0.072	0.734
Creatinine Clearance	-0.001	0.996
24hr.pro	-0.214	0.305
C3	-0.358	0.079
C4	0.401	0.047
Anti-dsDNA	-0.09	0.67

Table 5. Genotype frequency in the two groups studied

Genotype	Group *I* (n=15)	Group II (n=25)	p-value
AA (n= 2)	0 (0%)	2 (8%)	
AC (n= 20)	7 (46.7%)	13 (52%)	0.444
CC (n= 18)	8 (53.3%)	10 (40%)	

Data are expressed as a number (%).

Table 6. Alleles' frequency in the two groups studied

Alleles	Group *I* (n=15)	Group II (n=25)	p-value
A (n= 24)	7 (23.3%)	17 (34%)	0.313 (NS)
C (n= 79)	23 (76.7%)	33 (66%)	

Data are expressed as a number (%).

Table 7. Comparison of mean values of IL10, creatinine clearance, and 24-hour protein measured before and after treatment in group II

Variables	Before (n= 25)	After (n= 25)	p-value
Serum IL10 (U/ml)	15.24 ± 39.87	3.78 ± 4.05	0.165
Cr. Clearance (m/min.)	68.05 ± 24.28	67.80 ± 28.66	0.947
Protein 24 hrs (mg/day)	2587.24 ± 2439.54	1735.96 ± 1595.03	0.015

Data are expressed as mean ± SD.

Table 8. Comparison of mean values of IL10 measured before and after treatment in cases group (group *I*)

Variables	Active (n= 14)	Chronic (n= 6)	p-value
Serum IL10 before treatment (U/ml)	9.41 ± 13.60	4.73 ± 5.33	0.431 (NS)
Serum IL10 after treatment (U/ml)	3.56 ± 4.44	2.78 ± 2.75	0.700 (NS)

Data are expressed as mean ± SD.

4. Discussion

SLE is a systemic autoimmune disease characterized by autoantibodies, B cell hyperactivity, and immune complex formation (9). Glomerulonephritis is a frequent and often severe feature and is one of the major determinants of poor outcomes. Therefore, reliable markers for diagnosing and monitoring lupus nephritis are critically important. IL10 enhances B cell survival, proliferation, and antibody production, and these effects appear to play a role in autoimmune diseases (10). High IL10 expression and the corresponding IL10 alleles have been suggested to play a causal role in SLE (11), and polymorphisms of IL10 contribute—at least in part—to the genetics involved in SLE (12). Genetics could account for up to 75% of the IL10 production (13). The promoter of the IL10 gene has been shown to be highly polymorphic. Studies seeking to find an association between IL10 promoter polymorphisms and SLE have yielded varying results among different populations (14). For the most frequently studied 1082/-819/-592 SNPs, no association with SLE incidence has been found in Chinese, Dutch, or British populations (15), and no statistical difference has been found in the distribution of IL10-592 genotypes between lupus nephritis patients and those without renal involvement, suggesting that the -592 polymorphism in the IL10 gene may not be associated with lupus nephritis susceptibility.

Our study showed an increase in serum IL10 in lupus nephritis patients compared to those without renal involvement (without statistical significance), which concurs with the finding of Lit et al. (16). This proves the importance of IL10 in the pathogenesis of lupus nephritis. In addition, no statistically significant difference emerged in the level of serum IL10 among different pathological classes of our study or between active and chronic cases (which might be due to the small sample size). No statistically significant difference was found in the distribution of AC and CC genotypes between those with renal involvement and without. The CC genotype was more frequent in active cases with no statistical significance. In addition, no significant difference occurred in SLEDAI, anti DNA, proteinuria, hematuria, or casts in the different genotypes, which is contrary to Zhuet et al.'s (17) finding that patients with class IV had a higher frequency of AC/CC genotypes than those with class V lupus nephritis (genetic factor) contributing to the glomerular lesions in patients with lupus nephritis. Regarding the influence of IL10-592 A/C polymorphism on serum IL10 levels in lupus nephritis patients, no evidence of genetic regulation was reported in our study; however, its effect on renal lesions may affect the local IL10 levels in the glomeruli, not in the serum IL10, or perhaps be linked with some other genes that play a role in the pathological lesions of glomeruli. Anti DNA antibodies may play a role; however, our results show a negative correlation between their level and renal cases.

5. Conclusions

In conclusion, the findings of this study showed that IL10 gene -592 A/C polymorphism, although not associated with lupus nephritis susceptibility, does have an impact.

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Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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