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IL-10 Human Gene Polymorphism and Clinicopathological Diversity and Treatment Response in Egyptian Patients with Lupus Nephritis

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Abstract

Background and Aim of Study

Interleukin-10 (IL-10) is an important immunoregulatory cytokine. Many studies suggesting that a genetically controlled high innate IL-10 production may predispose to systemic lupus erythematosis (SLE) development. The aim of our study was to investigate IL-10 gene -592 A/C polymorphism in Egyptian patients with SLE and lupus nephritis (LN) and evaluate the role of IL-10 in the pathogenesis and clinical/pathological diversity of LN.

Patients and Methods

The study was conducted on 64 patients with SLE. Patients were divided into LN group [group 1; 42 patients with mean age 29.63 ± 8.91 yrs] and non nephritis group [group 2; 22 patients with mean age 31.81 ± 0.20yrs]. The IL-10 gene polymorphism of –592 A/C was determined by polymerase chain reaction and restriction fragment length polymorphism in LN and non LN patients.IL-10 was determined by ELISA. Frequencies of the genotypes were compared between LN and non LN patients and among LN patients with different pathological classes. The clinical and pathological characteristics of the patients with different genotypes were also analyzed. Patients of group1 were followed up for 6 months and 24 hrs urinary protein, Anti-ds DNA and IL-10 were measured after 6 months for group 1 to determine responders therapy.

Results

There was significant increase in serum level of IL-10 (p=0.0161) in (group 1) compared to (group 2) and significant positive correlation between serum IL-10 and SLEDAI (r=0.576,p=0.032) in group1.There was no significant differences in the distribution of the IL-10 -592 genotypes, or the alleles frequencies between (group 1) and (group 2).There was no significant difference between AC/CC and AA genotypes with disease activity(SLEDAI), proteinuria, hematuria, anti-ds DNA and IL-10 in (group 1).There was no significant difference in the distribution of AC and CC genotypes among different LN classes. There was significant decrease in serum IL-10 (p=0.0039), anti- ds DNA(P=0004), 24 hrs urinary protein (p=0.042), and SLEDAI (p=0047) after 6 months of therapy.

Conclusion

The IL-10 gene -592 A/C polymorphism not associated with LN susceptibility or serum IL-10 levels. IL-10 gene polymorphism may or may not play a role in the clinical and pathological diversity of LN and multiple factors are most likely responsible. IL-10 gene polymorphism may act indirectly through linkages with some other genes that play a role in the pathological lesions in lupus nephritis.

Keywords: Anti-ds DNA; IL-10 genotypes; Lupus nephritis; SLEDAI

Introduction

IL-10 is an important immunoregulatory cytokine that inhibits T cell function by suppressing the expression of proinflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, and IL- 12 [1]. It also inhibits antigen presenting cells by downregulating major histocompatibility complex class II (MHC-II) and B7 expression [1,2]. In addition to these inhibitory actions, IL-10 promotes B-cell-mediated functions, enhancing survival, proliferation, differentiation, and antibody production [3].

Hence, increased production of IL- 10 could thus explain B cell hyperactivity and autoantibody production, two main features of the immune dysregulation in systemic lupus erythematosis (SLE). In fact, elevated levels of this molecule have been currently reported in SLE patients, frequently associated with indicators of disease activity [4]. Moreover, it has been demonstrated that IL-10 plays an important role in murine lupus. Ishida et al reported that continuous administration of anti IL-10 antibodies in the murine lupus model *New Zealand black/white (NZB/W)F1* delayed the onset of autoimmunity and improved the survival rate from 10 to 80% [5]. Interestingly, Llorente et al. demonstrated that constitutive IL-10 production by monocytes and B cells in healthy members of multicase families with SLE was significantly higher than that of healthy unrelated controls, but was similar to that of SLE patients, thus suggesting that a genetically controlled high innate IL-10 production may predispose to SLE development [6].

Aim of the Study

The aim of our study was to investigate the distribution of IL-10 gene -592 A/C polymorphism in Egyption patients with SLE and lupus nephritis (LN) and evaluate the role of lL-10 in the pathogenesis and clinical/pathological diversity of LN.

Patients and Methods

The study was conducted on 64 patients with SLE who fulfilled at least four of American college of Rheumatology criteria for classification of SLE. LN diagnosed by presence of persistent proteinuria or hematuria [7]. Patients were divided into LN group [group 1; 42 patients with mean age 29.63 ± 8.91 yrs] and non nephritis group [group 2; 22 patients with mean age 31.81 ± 0.20yrs]. SLE patients with proteinuria other than LN as pregnancy and fever or patients with impaired renal function due to any other cause than LN as diabetic nephropathy and patients with history of renal transplantation or HCV&HBV and other connective tissue diseases other than SLE were excluded from the study. Kidney biopsy was performed to LN group and classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of LN [8].Written informed consent was obtained from all participants in the study.

All patients of both groups were subjected to the following.

I) Full history taking and complete clinical examination

II) Laboratory Investigations:

i) Sampling:

Venous blood samples were collected from patients after proper disinfection.

(a) 2 ml on EDTA for CBC.

(b) 2ml on another EDTA for real time PCR.

(c) 3ml of blood in a plain glass tube left to clot at room temperature for 30 minutes then centrifuged for 10 minutes to obtain serum for chemical and immunological tests.

ii) Routine laboratory investigations as Complete blood count (CBC), blood urea, serum creatinine, urine analysis, erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP) were measured for the studied patients

iii) Immunological profiles:

- ANA (Antinuclear Antibody) by indirect immune-flourescence
- Anti-ds DNA by solid-phase enzyme immunoassays kits.
- C3 and C4 were measured using BN ProSpec Nephelometery.(106)

Determination of –592 A/C polymorphisms in the IL-10 gene promoter

Genomic DNA was extracted from whole blood using a phenol chloroform extraction method. The IL-10 gene polymorphism of –592 A/C was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses.

The primer sequences were:

5` TCC AGC CAC AGA AGC TTA CAA C 3` (forward)

5` AGG TCT CTG GGC CTT AGT TTC C 3` (reversed)

PCR was performed on a Gene- Amp PCR System 2700 (Applied Biosystems, USA) in the following conditions: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 62°C for 30 seconds, and 2°C for 30 seconds; followed by a final extending step at 72°C for 5 minutes.

The PCR product was digested for 4 hours at 37°C with the restriction enzyme Rsa I (New England Bio- Labs, USA). The genotypes of IL-10 –592 A/C were distinguished by separation of the fragments on a 2.5% agarose gel and visualized under ultraviolet light.

Detection of Serum of IL-10 Level

IL-10 was determined by ELISA using an ELISA kits (AviBion human IL-10 ELISA kit).

Renal Biopsy

All SLE patients with clinical and laboratory evidence of renal involvement will be subjected to renal biopsy. The renal cores will be examined for assessment of the activity and chronicity indices and will be examined and classified into different subclasses of LN that will be performed according to the scheme of the (ISN/RPS) 2003 classification of LN [8].

Follow Up

Twenty four hrs urinary protein, anti-ds DNA and IL-10 will be followed up after 6 months for group 1 to determine responders to a fixed protocol of therapy formed of corticosteroid, azathioprine and cyclophosphamide.

Statistical Analysis

Results were expressed as means ± standard deviation of the means (SD) or number (%). Comparison between different parameters in the two studied groups was performed using unpaired two-tailed Student's *t*-tests (Graphpad Quick Calcs). Comparison between categorical data was performed using Chi square test. Correlation between different parameters in the cases group was performed using Pearson correlation. Statistical analysis was performed with the aid of the SPSS computer program (version 12 windows).The data were considered significant if *p-value* was equal to or less than 0.05 and highly significant if *p-value* < 0.01.

Results

Demographic and clinical characteristics of the studied groups are shown in (Table 1). Laboratory parameters of the studied groups are shown in Table 2, where, there was statistically significant increase in anti-ds DNA, in group 1 compared to group 2 (p=0.0006).On the other hand there was statistically significant decrease in C3 (p=0.0001) and C4 (p=0.0002) in group 1 compared to group 2 (Table 2).

There was a statistically significant increase in the mean value of systemic lupus erythematos is disease activity index (SLEDAI) (P=0.0235) and SLEDDI in group 1 compared to group 2 (P=0.0001) (Table 3).

There was statistically significant increase in serum level of IL-10 (p=0.0161) in group 1 compared to group 2 (Table 4).

There was a statistically significant negative correlation between serum IL-10 level and serum C3 (r = -0.512, p= 0.024), and a statistically significant positive correlation between serum IL-10 and SLEDAI (r=0.375, p=0.032). There was no statistically significant difference in the distribution of the IL-10-592 A/C genotypes, or the alleles frequencies between group 1 and group 2 (Table 5).

There was no statistically significant difference between AC/CC and AA genotypes with different clinical and laboratory parameters in group 1 (Table 6).

Table 1:Demographic and clinical characteristics of the studied groups

Variable	Group 1(n=42)	Group 2(n=22)	P values
Age	29.63 ±8.91	31.81 ±0.20	0.2574(NS)
Gender(M/F)	4/36(10%/90%)	1/19(5%/95%)	0.865(NS)
Duration of disease(yrs)	6.23±3.00	5.12±1.83	0.1182(NS)
Oedema of LL	29(69.05%)	0(0%)	0.001**
Hypertension	27(64.3%)	0(0%)	0.001**
Artheritis	26(61.9%)	13(59.09%)	
Skin rash	23(54.78%)	10(45.5%	

Data are expressed as mean +SD or number (%).NS=Non significant=p>0.05. **P<0.01=highly significant

Table 2:Laboratory parameters in the studied groups.

Variables	Group 1(n=42)	Group 2(n=22)	P value
S.creatinine(mg/dl)	2.45±2.26	0.80±0.15	0.0012**
BL.urea(mg/dl)	66.05±6 5.02	29.30±9.08	0.0001**
24 hrs urinary protein(g/day)	2616.92±2321.38	131.876 ±0.13	0.0001**
Urine analysis Hematuria Casts	 24(57.14%) 29(69.05%)	 0(0%) 0(0%)	 0.001** 0.001**
CRP(mg/dl)	9.14±8.34	8.23±7.13	0.6652(NS)
ESR(mm/hr)	96.51±2 9.36	63.11±38.79	0.0003**
C3(g/l) C4(g/l)	0.75±0.52 0.23±0.44	1.23±0.21 0.74±0.57	0.0001** 0.0002**
ANA	1/620	1/80	0.001**
Anti ds DNA(IU/ml)	174.11 ±149.52	58.21 ±23.47	0.0006**

Data are expressed as mean +SD or number(%).NS=Non significant=p>0.05. **P<0.001=highly significant

Table (3): Comparison between SLEDAI and SLEDDI in the studied groups.

Variables	Group 1(n=42)	Group 2(n=22)	P value
SLEDAI	14.60±8.63	10.17±3 .11	0.0235**
SLEDDI	0.90±0.54	0.12±0.33	0.0001**

SLEDAI = systemic lupus erythematosis disease activity index
Data are expressed as mean+SD. **P<0.01=highly significant

Table (4): Comparison between IL-10 in the studied groups.

Variable	Group 1(n=42)	Group 2(n=22)	P value
Serum IL-10(U/ml)	21.13±3 2.17	3.96±5.81	0.0161*

Data are expressed as mean+SD. *P<0.05=significant

Table (5): Distribution of IL-10-592 A/C gene polymorphism and alleles frequency in the two studied groups.

A/C gene polymorphism	Group 1(n=42)	Group 2(n=22)	P value
AA(N=6)	6(14.3%)	0(0%)	0.182(NS)
AC(N=31)	20(47.6%)	11(50%)	
CC(N=27)	16(38.1%)	11(50%)	
Alleles	Group 1(n=84)	Group 2(n=44)	
A(n=45)	33(39.3%)	12(27.3%)	0.153(NS)
C(n=83)	51(60.7%)	32(72.7%)	

Data are expressed as number(%).NS=P>0.05=non significant.

Table (6): Clinical and laboratory parameters in LN patients with different genotypes.

Variables	AA(n=6)	AC+CC(n 36)	P value
24 hs urinary protein	1592.47±1238.13	2783.46±2412.34	0.247(NS)
Urinary cast(+ve)	3(50%)	22(61.1%)	0.567(NS)
Urinary RBCs(+ve)	3(50%)	14(38.9%)	0.676(NS)
SLEDAI	11.34±5.13	12.14±8.26	0.8204(NS)
Anti-ds DNA	171.65±122.11	173.48±151.21	0.978(NS)
Serum IL-10	7.15±5.65	23.51±43.48	0.368(NS)

Data are expressed as number(%).P>0.05=NS=non significant

Renal Pathology Data

Thirteen patients (30.95%) were classified as class III LN, 17 patients (40.5%) were classified as class IV LN, 7 patients (16.6%) were classified as class V, 4 patients (9.5%) were classified as class IV+V and 1 patient (2.4%) was classified as class VI. According to activity and chronicity indices [8], class IIIa and IVa are classified as active classes while class IIIc and IVc are classified as chronic classes.

There was no statistically significant difference between AC/CC and AA genotypes with different pathological classes of LN (Table 7).

Follow Up Data

Eight patients of group 1 didn't complete the follow up. There was statistically significant decrease in serum anti-ds DNA (p=0.0004), serum IL-10 (p=0.0039) (Figure 1), 24 hrs urinary protein (p=0.0420), and SLEDAI (p=0.0047) after 6 months of therapy in group 1 (Table 8).

However, there was no statistically significant difference in the level of IL-10 before and after treatment among the LN pathological classes (Table 9).

Table (7) : Comparison between CC and AC genotypes in different pathological classes:

	Class 111(n=12)	Class 1V(n=16)	Class V(n=6)	P value
AC(n=17)	8	7	2	0.0735(NS)
CC(n=13)	4	9	4	

NS=P>0.05=non significance. Data are expressed as numbers%

Table (8) : Comparison between 24 hrs urinary protein, anti ds DNA and IL-10 before and after treatment in (group 1).

Variables	Before(n=34)	After(n=34)	P value
24 hrs urinary protein(mg/day)	2616.92 ±2321.38	1621.81±1562.11	0.0420*
SLEDAI	14.60±8.63	9.32±6.03	0.0047**
Serum IL-10(U/ml)	21.13 ±32.17	4.46±4.23	0.0039**
Anti ds DNA(U/ml)	174.11 ±149.52	76.28±27.31	0.0004**

Data are expressed as mean+SD. *P<0.05=significant. **P<0.01=highly significant

Table (9) : Comparison between mean values of serum IL-10 before and after treatment in different pathological classes in (group 1) according to ISN/RPS classification.

	Class 111(n=13)	Class 1V(n=17)	Class V(n=7)
Serum IL-10 before treatment	22.30±49.36	10.24±13.78	44.20±75.12
Serum IL-10 after treatment	4.54+4.12	5.38+5.44	5.16+5.12
P value	0.2084(NS)	0.1857(NS)	0.1952(NS)

P value>0.05=NS=non significant

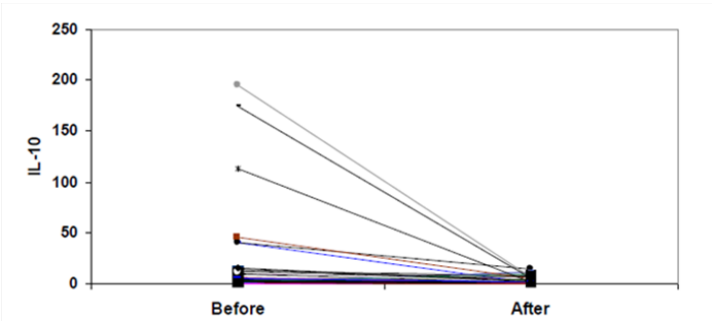

Figure 1: Serum IL-10 (Unit/ml) before and after treatment in group1 (p value=0.0039)

Discussion

We did not find statistically significant difference in the distribution of different IL-10 –592 genotypes between LN patients and those without renal involvement, suggesting that the –592 polymorphism in the IL-10 gene may not be associated with LN susceptibility.

IL-10, is a cytokine, which is produced primarily by monocytes and to a lesser extent by lymphocytes, that has pleiotropic effects in immunoregulation and inflammation [9]. The IL-10 gene is located on chromosome-1, and its receptor is located on chromosome 11 [10].

High IL-10 expression and the corresponding IL-10 alleles have been suggested to play a causal or exacerbating role in SLE [11]. A 40-fold increased risk for developing SLE was identified in individuals with particular alleles of both the IL-10 and bcl-2 genes , and it has been considered that polymorphisms of IL-10 contribute, at least in part, to the genetics involved in SLE [12].There is more consensus concerning the role played by IL-10 levels are consistently high in the serum of patients with SLE, and anti-IL-10 antibodies ameliorate disease in murine models of SLE [5].)

Several studies have shown that unaffected family members of SLE patients produce high levels of IL-10.The implication of a heritable genetic basis for IL-10 production is supported by the concordance of IL-10 production in monozygotic twins, which suggests that genetics could account for up to 75% of IL-10 production [6]. Indeed, the high IL-10 production associated with autoimmune diseases including rheumatoid arthritis (RA) and SLE may be a genetic risk factor for disease susceptibility [13].The basis for heritable differences in IL-10 production is not known [14].

Although the kidney is one of the major target organs involved in SLE immunological damage, there have been few studies concentrating on the association between IL-10 and LN [14].

The role of IL-10 in mouse models of lupus may not reflect the full extent of IL-10 involvement in the pathogenesis of SLE in man because mouse B cells do not respond to IL-10 as human B cells do. While IL-10 dramatically increase proliferation, differentiation, and antibody production by anti-CD40 stimulated human B cells in culture, no such activity is seen in mouse splenic B cells [15].

The present study showed that serum IL-10 were statistically significant higher in the LN group compared to non-LN group which was also observed by Lit et al.[16].These results indicate that dysregulation of IL-10 may play an important role in the pathogenesis and development of LN.

In our study,IL-10 was found to correlate positively with SLEDAI and negatively with C3 which is similar to the results of another study that noted that increased levels of IL-10 have been found in the serum of patients with SLE which correlate with disease activity using SLEDAI [17].

The association of IL-10 with glomerular hypercellularity has been reported by several studies. IL-10 can induce significant mesangial cell proliferation in vitro and in vivo [18], and inhibition of IL-10 by the immunomodulator AS101 reduced mesangial cell proliferation in experimental mesangioproliferative glomerulonephritis [19]. In situ hybridization and immunohistochemistry revealed that IL-10 mRNA and protein,expression were significantly upregulated in the glomeruli with marked proliferative responses and in acute phases of microscopic polyangiitis [20].

There was a significant racial variation in the distribution of the –592 A/C polymorphism of the IL- 10 gene. In comparison with the significant difference between SLE patients with and without renal involvement in the Hong Kong Chinese population [21], our study revealed that, there was no statistical difference in the distribution of the IL-10 –592 genotypes between LN patients and those without renal involvement, suggesting that the –592 polymorphism in the IL-10 gene may not be associated with LN susceptibility [14].

The current study showed that in patients with AC/CC genotypes revealed no statistically significant increase in SLEDAI, anti-ds DNA, or urinary casts. In opposition to our results, Zhu and his collegoues [14] observed that the SLEDAI, urinary casts, anti-dsDNA were all statistically significant higher in patients with AC/CC genotypes than those with AA genotype.

The IL-10 –592 A/C polymorphism has been shown to influence IL-10 secretion in studies in vitro and the A allele was associated with lower IL-10 release in lipopolysaccharide-stimulated peripheral blood mononuclear cells (PBMCs) [22]. In our study, there was no statistically significant difference in the level of IL-10 in AC/CC genotypes compared to AA genotype which is similar to another study [14].

It still remains to be determined how the genetic polymorphism not associated with serum IL-10 level may have impact on renal lesions of LN. One study showed that the 592 C allele was associated with a higher frequency of positive anti-dsDNA, which have been considered to be responsible for the initiation of LN [14].This is in opposition to our results as 592 C allele was not associated with a higher frequency of positive anti-dsDNA.

We also found that there was no statisticaly significant increase in SLEDAI, anti-dsDNA, proteiuria, hematuria, and casts in AC/CC genotypes compared to AA genotype (which may be attributed to small sample size as AA genotype was observed in only 6 patients) . In opposition to our results, another study noted that patients with AC/CC genotypes patients showed statisticaly significant increase in SLEDAI, anti-dsDNA, proteiuria, hematuria, and casts compared to AA genotype spatients [14].)

As regard the association of IL-10 gene polymorphism with different renal pathological classes of LN, our finding that there was no statistically significant difference in the distribution of AC (p=0.079) and CC genotypes (AA was not included due to its small number) among different classes of LN (III, IV and V). Moreover, the increase in the frequency of CC genotype in patients with active classes (83.33%) compared to AC (73.33%) genotype did not reach the statistical significance. However, other study was done by Zhu et al. [14] found that patients with class IV LN had a higher frequency of AC/CC genotypes than those with class V LN suggesting that genetic factors may contribute to the glomerular lesions in patients with LN [14]. This result revealed that the IL-10 gene polymorphism may play a role in diverse renal pathological changes in LN patients and that those carrying a high-IL-10-producing allele were more likely to have diffuse proliferative lesions in the kidney. In fact, the pattern of immune complex deposition of each pathological class of LN appears to be different. It is possible that the IL-10 gene polymorphism may influence the renal lesions through anti-dsDNA, but the mechanism is still to be identified. Secondly, the IL-10 gene polymorphism might possibly impact on the local IL-10 level in the glomeruli, other than the serum IL-10 level, that could be responsible for the renal lesions in LN and difference among different pathological classes [15]. It has been shown that IL-10 could act as a monocyte chemotactic factor in the presence of immune complex correlated with enhanced antibody-dependent cell mediated cytotoxicity (ADCC).The degree of immune cell infiltrating and glomerular hypercellularity, therefore, can be influenced by renal IL-10 levels [20].

It is also possible that the IL-10 gene polymorphism may act indirectly through linkages with some other genes that play a role in the pathological lesions in the lupus glomeruli. Further studies concerning these aspects are

to be carried out to identify the role of the IL-10 gene polymorphism in the renal pathology of LN. Furthermore, we cannot neglect the fact that the pathological type in a LN patient may not always be invariable and we need to follow up these patients to collect more evidence, especially the pathological diagnosis from repeated renal biopsies [14].

During follow up of patients we observed that there was a statistical significant decrease in serum IL-10 level after the period of follow up. However, there was no statistically significant difference between the level of IL-10 and different pathological classes and also there was no statistical significant difference between the level of IL-10 between active and chronic classes of renal biopsy.

Conclusion

IL-10 gene –592 A/C polymorphism, though not associated with susceptibility to LN or the difference of the patients" serum IL-10 levels, may or may not play a role in the clinical and pathological diversity of LN and multiple factors are most likely responsible. IL-10 gene polymorphism may act indirectly through linkages with some other genes that play a role in the pathological lesions in lupus glomeruli.

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