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Association of Interleukin-6 and Interleukin -10 Gene Polymorphisms with Susceptibility and Clinical Features of Systemic Lupus Erythematosus in Bangladeshi Population

Nasrin Nahar[1], Raisa Enayet Badhan[*,2], Sharmeen Ahmed[3], Rehana Razzak Khan[4], Shanjida Khondakar Setu[5]

Abstract

Background: Several cellular and cytokine abnormalities have been detected in Systemic lupus erythematosus (SLE) throughout the last three decades. Single nucleotide polymorphisms (SNPs) in cytokine genes have been shown to have an important role in the overall pathogenesis of SLE. Aim of the study was to demonstrate the association of IL-6 and IL-10 gene polymorphisms with susceptibility and clinical features in Bangladeshi SLE patients.

Methods: According to 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria, total 75 SLE patients and 75 healthy controls were enrolled in this study. IL-6 and IL-10 gene polymorphisms were detected by polymeric chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. All statistical analysis was performed using the statistical pakage for social science (SPSS) program, 26 version.

Results: GG genotype and G allele of IL-6 (-174G/C) was higher in case compared to control group. The homozygous AA genotype, combined CA/AA genotype and A allele of IL-10 (-592C/A) were found significantly higher in patients than in control. The TC/CC and C allele of IL-10 (-819T/C) are found to be significant in patients than in control. TC/CC was found to be significantly higher in patients with oral ulcer as compared with patients having TT genotypes.

Conclusion: It can be concluded that IL-6 (-174G/C) and IL-10 (-592C/A), IL-10 (-819T/C) gene polymorphisms may play a role in SLE susceptibility in Bangladeshi population. The TC/CC genotype of IL-10 (-819T/C) gene appears to be associated with oral ulcer in SLE patients.

Keywords: IL-6, IL-10, SNP, PCR-RFLP

Background

SLE is an autoimmune illness that affects several organs [1]. This autoimmune illness is distinguished by a loss of tolerance to self-antigens, which results in the activation of auto reactive B and T cells, the production of autoantibodies against nuclear antigens, and the deposition of immune complexes in various tissues [2]. The incidence and prevalence of SLE vary greatly across populations and races. The prevalence of lupus is estimated to be 51 per 100,000 people in the United States. According to data from 24 Asian countries, the prevalence of SLE is between 30 and 50 per 100,000 people [3]. It is worth noting that, while SLE occurs worldwide, it is more

Affiliation:

[1]Lecturer, Department of Microbiology, Sher-E-Bangla Medical College, Barisal, Bangladesh

[2]Medical officer, Department of Microbiology, National Institute of Burn and Plastic Surgery, Dhaka, Bangladesh

[3]Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

[4]Assistant professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

[5]Associate professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

***Corresponding author:**
Raisa Enayet Badhan, Medical officer, Department of Microbiology, National Institute of Burn and Plastic Surgery, Dhaka, Bangladesh.

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frequent in some nations, and certain ethnic groups tend to be more prone to developing the condition than others. Various studies indicated the prevalence of SLE to be 30-70/100000 population in China and 3.2/100000 in India [3]. The frequency of SLE in Bangladesh is unknown [4]. SLE affects genetically susceptible individuals who have undergone specific environmental or stochastic triggers. The intricate combination of genetics, environment, and hormones leads immune control to fail, resulting in the creation of autoantibodies, inflammation, and damage of the ultimate organs [5]. Autoantibodies against a wide range of nuclear and cell surface autoantigens produce immune-mediated tissue damage, which manifests clinically as arthritis, vasculitis, nephritis, mouth ulcers, skin rash, and photosensitivity [6].

IL-6 plays an important role in the pathophysiology of human SLE by hyperactivating B cells, resulting in increased autoantibody synthesis, which is a major immunological abnormality in SLE [7]. The IL-6 gene has been identified on chromosome 7's short arm, namely at positions 21-15 [8]. Overexpression of IL-6 in SLE could be caused by a high concentration of up-regulating factors and polymorphisms in gene regulatory regions [9]. It lacks any common polymorphisms inside coding region; hence research has been concentrated on promoter polymorphisms.

The IL-6 promoter variations -597G/A, -572G/C, and -174G/C have been demonstrated to alter its transcription and secretion. The common SNP-174 G/C has since become the most extensively researched locus in relation to IL-6 [10]. This polymorphism has a substantial association with SLE risk in the European population [11]. The IL-6 'G' allele at the -174 location of the 5'promoter region is linked to an increased risk of SLE in the south Indian population [12]. IL-10 is a significant pleiotropic cytokine with both anti-inflammatory and stimulatory properties [13]. Although the significance of IL-10 in the pathophysiology of SLE is uncertain, SLE patients produce more IL-10. Furthermore, overproduction of IL-10 impacts the manufacture of autoantibodies, which happens in SLE patients [14]. The clinical improvements in SLE patients following the administration of an anti-IL-10 monoclonal antibody confirm IL-10's essential role in the development of SLE [15,16]. A number of observational studies have been undertaken to demonstrate the relationship of IL-10 gene polymorphisms with SLE susceptibility, although the findings are inconsistent. The IL-10 gene has been identified on human chromosome 1, between 1q31 and 1q32; this locus is recognized to be one of the key SLE susceptibility loci [17]. The IL-10 promoter is highly polymorphic, with two microsatellites (MS) (IL10R and IL10G) and three SNPs. It has been shown that promoter polymorphisms in the IL-10 gene are connected to genetic predisposition to SLE. SNPs of the proximal regions (-1082G/A, -819T/C, -592C/A) have been reported to influence disease susceptibility among different populations [18]. A meta-analysis of the IL-10-1082G/A polymorphism demonstrates a link between susceptibility to SLE and the IL-10-1082G allele in Europeans but not in Asians [19]. The -1082G/A, -819T/C, and -592C/A polymorphisms are associated with SLE susceptibility in a Caucasian population [20]. The IL-10-592-A allele has been linked to the development of lupus nephritis in Chinese SLE patients [21]. The IL-10 -819T/C polymorphism and the C allele are widely distributed in Asian SLE patients [22]. The connection between the aforementioned cytokine genes and susceptibility to SLE in several research was not consistent, presumably because to ethnic and racial variances. Aim of this study was to investigate the potential association of four SNPs at IL-6 (-174G/C) and IL-10 (-1082G/A, -592C/A, -819T/C) with the susceptibility and severity of SLE in Bangladeshi population. The results of the study might be proven to be important to understand the role of cytokine gene polymorphism in SLE patients which will help in better understanding of disease pathogenesis, host immune response, susceptibility and to develop the therapeutic strategies for SLE.

Methodology

This was a case control study was conducted from March 2021 to February 2022 in the department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka.

Study population

A total of 75 SLE patients attending both outdoor/ SLE clinic and the department of Rheumatology, BSMMU, Shahbag, Dhaka were selected as cases. Healthy control groups were selected from the laboratory and nursing stuff, post graduate students and general people of same geographical area without any diagnosed connective tissue diseases.

Inclusion criteria

Systemic Lupus Erythematosus patients diagnosed by expert Rheumatologists of the department of Rheumatology, BSMMU fulfilling "2019 EULAR/ACR classification criteria for SLE" were enrolled in the study as cases. On the other hand, person without SLE and who were anti neuclear antibody (ANA) test negative were included as controls.

Exclusion criteria

Patients having overlap syndrome in association with SLE, patients with pregnancy, with acute infection, severely ill patients and person unwilling to participate are excluded from case group. On the other hand, person having family history of SLE or other rheumatic diseases, pregnant woman, acute infection and person unwilling to participate were excluded as controls.

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Sample size: A total of 75 cases and 75 controls were included in this study.

Sampling procedure: Purposive sampling.

Laboratory procedure

Detection of IL-6(-174G/C) and IL-10(-1082G/A, -819T/C, -592C/A) gene polymorphism by PCR-RFLP method

PCR-RFLP for IL-6 and IL-10 gene polymorphism were done. DNA was extracted from peripheral venous blood. PCR assays was performed in PCR laboratory of Department of Microbiology and Immunology.

Method of DNA extraction and DNA concentration determination

Genomic DNA extraction from peripheral venous blood was done according to manufacturer's instruction (Genomic DNA extraction Kit, Anatolia Geneworks, Bosphore, Turkey). Volume of clinical sample for DNA extraction was 200 µl. The concentration of DNA was measured by spectrophotometric assay performed using a Nanodrop 2000 spectrophotometer (Thermo Fisher scientific, Waltham, MA, USA) according to manufacturer's instruction.

IL-10 Genotyping

SNPs at the positions 819 T/ C, 1082 A/G and 592 C/A for IL-10 PCR was performed using a 20 µl reaction mixture that included 15 µl master mix (Takara Bio Inc.), 3 µl nuclease free water, 1 µl each set of primers. Then 5 µl DNA was added. For 819C/T, forward and reverse primers were 5AGTAAGGGACCTCCTATCCAGCC3 and 5CTCAAAGTT CCCAAGCAGCC3. For 1082 G/A, forward and reverse primers were 5CACACAAAT CCAAGACAACACTACTAAGGCTTCTTTCTGA3 and 5ATAGTGAGCAAACTGAGGCACAGAG3. For 592 C/A, forward and reverse primers were 5GGTGAGCACTACCTGACTAGC3 and 5CCTAGG TCACAGTGACGTGG3 [23]. Amplification were done in proflex PCR system (Applied Biosystems, Thermo fisher scientific, USA) as follows: For 819C/T, initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 35 seconds and final extension at 72°C for 5 minutes. For 1082 G/A, initial denaturation at 94°C for 10 minutes, 40 cycles of 94°C for 30 seconds, 60°C for 60 seconds, 72°C for 10 seconds and final extension at 72°C for 1 minute. For 592 C/A, initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 5 minutes. RFLP was performed by restriction enzyme (MslI for 819C/T, AcuI for 1082 G/A and RsaI for 592 C/A) according to manufacturer guidelines (New England Biolabs, Ipswich, MA, USA) at 37°C overnight then followed by electrophoresis on 2% agarose gel with ethidium bromide.

For 819T/C T allele, PCR product were uncleaved 520bp. For C allele, PCR product were cleaved 279 and 241bp [23]. **(Figure1a)**

For 592 C/ A C allele, PCR product were uncleaved 412bp. For A allele, cleaved 176bp and 236bp [23]. **(Figure1b)**

For 1082 G/A G allele, PCR product were uncleaved 331bp. For A allele, were cleaved 289bp and 42bp [23]. **(Figure1c)**

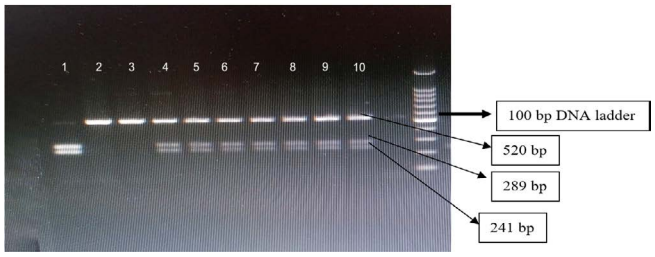

Figure 1a: PCR-RFLP analysis of IL-10 (-819T/C) polymorphism by Msl1 enzyme agarose gel electrophoresis.

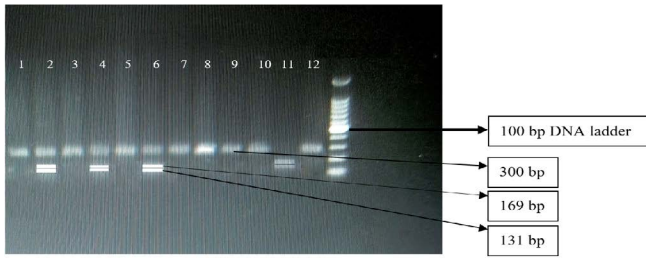

Figure 1b: PCR-RFLP analysis of IL-10 (-592C/A) polymorphism by Rsa1 enzyme agarose gel electrophoresis.

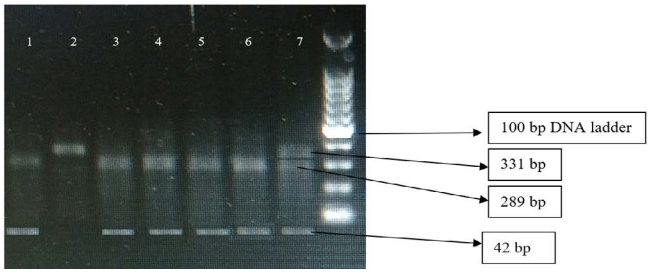

Figure 1c: PCR-RFLP analysis of IL-10 (-1082 G/A) polymorphism by Acul enzyme agarose gel electrophoresis.

IL-6 Genotyping:

For IL-6 (-174G/C) PCR was also performed using a 20 µl reaction mixture. Then 5 µl DNA was added. For -174G/C, forward and reverse primers were 5TTGTCAAGACATGCCAAAGTC3 and 5TCAGACATCTCCAGTCCTATA3 [1]. Amplification were carried out in proflex PCR system (Applied Biosystems, Thermo fisher scientific, USA) as follows: For -174G/C,

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initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 52°C for 45 seconds, 72°C for 45 seconds and final extension at 70°C for 5 minutes. RFLP was performed by restriction enzyme N1alll according to manufacturer guidelines (New England Biolabs, Ipswich, MA, USA) at 37°C overnight followed by electrophoresis on 2% agarose gel stained with ethidium bromide. PCR product were uncleaved 300bp. For C allele, PCR product were cleaved169bp and 131bp [1]. **(Figure 2)**

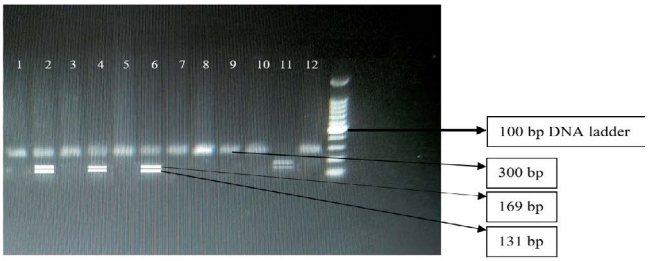

Figure 2: PCR-RFLP analysis of IL-6 (-174G/C) polymorphism by N1alll enzyme agarose gel electrophoresis.

Detection of serum level of IL-6: Determination of IL-6 level was done by Chemiluminescence Immunoassay (Bechman Coulter) according to manufacturer's instruction (ADVIA centaur IL-6 assay kit, Siemens).

Detection of serum level of Il-10: Stored serum from patients with SLE were used for determining the level of IL-10 using enzyme linked immunosorbant assay (ELISA) kit according to manufacturer's instruction (Elabscience, USA; catalog no: E-EL-H6154) .

Data analysis: Collected data was checked, edited and analysis was performed with SPSS software package version-26 (Strata Corporation, College station, Texas). Continuous parameters were expressed as mean±SD and categorical parameters as frequency and percentage. P value calculated by Mann-whitney U test to compare the serum cytokine levels in SLE patients and control. Comparison of serum level of cytokines of SLE patient group (mild to moderate group and severe group) was calculated by Mann-whitney U test. Association of SLEDAI-score with serum level of cytokines was calculated by Pearson correlation test. Association of genetic variations with disease susceptibility, with respect to healthy control and association of genetic variation with clinical manifestations was calculated by Chi-squire test. To see the difference between two groups, Man-Whitney U test was done. For all test a P value <0.05 was considered as statistically significant.

Results

The mean age of patient with SLE was (29.43±8.08) years. Out of 75 patients, 71(94.66%) were female and 4 (5.34%) were male with a female to male ratio of 17.75:1. Most of the patients (68.0%) had arthritis followed by rash (52.0%), lupus nephritis (33.3%), oral ulcer (30.7%) and hematological disorders (2.7%). The comparison of genotypic distribution and allelic frequencies of the IL-6 (174G/C) gene between case and control are summarized in **table 1**. The homogygous GG genotype was observed higher in SLE group (73.3%) compared to healthy control group (53.3%) (*P*= 0.011). The heterogyzous GC genotype was found significantly higher in healthy controls 33 (44.0%) compared to SLE group 20(26.7%) (*P*= 0.026). The homogygous CC genotype was found only in 2 (2.7%) healthy controls (*P*=0.154). The allelic frequency of G allele in SLE patients is 130 (86.7%) and in healthy control group is 113 (75.3%) (*P* =0.012). The allelic frequency of C allele was 20 (13.3%) and 37 (24.7%) in patients and in healthy control groups respectively. It indicates that G allele and GG genotype of IL-6 (174G/C) has got significant association with SLE. On the other hand, GC genotype may be associated with decreased susceptibility to SLE. Association of IL-6 (174 G/C) polymorphisms with clinical menifestations in SLE patients are shown in **table 2**. There is no significant association of IL-6(-174 G/C) with the clinical manifestation of SLE patients.

Table 1: Comparison of allele and genotype frequencies of IL-6(-174G/C) in SLE patients and controls (n=150)

IL-6 (174 G/C) Genotype and Allele	Case (n=75)	Control (n=75)	OR (95% CI)	*P*-value
GG	55(73.3%)	40(53.3%)	2.41(1.21-4.77)	0.011*
GC	20(26.7%)	33(44.0%)	0.462 (0.23-0.92)	0.026*
CC	0(0.0%)	2(2.7%)	-	0.154
G allele	130(86.7%)	113(75.3%)	0.47(0.26-0.86)	0.012*
C allele	20(13.3%)	37(24.7%)		

Note: *significant
P value reached from Chi-square test

Table 2: Association of IL-6 (174 G/C) polymorphisms with clinical manifestations in SLE patients (n=75)

Clinical manifestations	Genotype GG (n=55)	Genotype GC (n=20)	OR (95% CI)	*P*-value
Rash	32(58.2%)	7(35.0%)	2.58 (0.89-7.45)	0.076
Oral ulcer	19(34.5%)	4(20.0%)	2.11 (0.62-7.21)	0.227
Arthralgia	36(65.5%)	15(75.0%)	0.63 (0.20-2.0)	0.433
Lupus nephritis	17(30.9%)	8(40.0%)	0.67(0.23-1.94)	0.46
Hematological disorder	2(3.6%)	0(0.0%)	-	0.387

Note: *significant
P value reached from Chi-square test

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The allelic and genotypic frequencies of IL-10 (-1082 G/A), IL-10 (-592 C/A) and IL-10 (-819 T/C) are tabulated in **table 3**. The genotype distribution of IL-10 (-1082 G/A) showed that the genotype GG was 21.3% in patient group and 16.0% in healthy control group (*P*= 0.409). The heterozygous GA genotype were found 24.0% and 25.3% in patients and healthy control group respectively (*P*= 0.849). The homozygous AA genotype were 54.7% in SLE patients and 58.7% in healthy control groups (*P*= 0.621). The combined genotype GA/AA was 78.7% in case group and 84.0% in control group (*P*= 0.402). The A allelic frequency was 66.7% in patient group and 71.3% in healthy control group (*P*= 0.382). It indicates that no genotypic or allelic distribution are significantly associated with the susceptibility to SLE. The genotype distribution for the IL-10 (-592 C/A) gene polymorphism were significantly different between patients and healthy controls. The frequency of wild type genotype CC was found significantly higher (49.3%) in healthy controls compared to patient group (24.0%) (*P*= 0.001). The heterozygous combination CA was found 41 (54.7%) in SLE patients and 31 (41.3%) in healthy controls (*P*=0.102). The homogygous AA genotype was found 16 (21.3%) in patients and 7 (9.3%) in control group (*P*=0.041). The combined genotype CA/AA was significantly found higher (76.0%) in patients compared to healthy controls (50.7%) (*P*= 0.001). The A allelic frequency was 73 (48.7%) in SLE patients and 45(30.0%) in control groups (*P*=0.001). It indicates that AA genotype and combined CA/AA genotype and A allele are significantly associated with susceptibility to SLE and CC genotype may be associated with decreased susceptibility to SLE. The genotype distribution of IL-10 (-819T/C) showed that the frequency of wild type genotype TT was found higher in healthy control groups compared to patients (33.3% vs 18.7%) (*P*= 0.041). The heterogyzous TC genotype was found in 36 (48%) SLE patients and 35 (46.7%) in healthy control group (*P*=0.87). The homogygous CC genotype was found 25 (33.3%) in SLE patients and 15 (20.0%) in healthy control groups (*P*=0.065). The combined genotype TC/CC was found higher (81.3%) in case group compared to healthy control (66.7%) (*P*=0.041). The C allelic frequency was 86 (57.3%) in case group and 65 (43.3%) in control group (*P*= 0.015). It indicates that combined TC/CC genotype and C allele are significantly associated with susceptibility to SLE. On the other hand, TT genotype may be associated with decreased susceptibility to SLE.

Clinical manifestations of SLE patients with different IL-10-1082G/A genotypes are shown in **table 4**. There was no significant association of genotypic distribution with clinical manifestations in SLE patients. To examine the association of IL-10 (819T/C) gene polymorphism with the SLE phenotype, the combined genotype frequency distribution was stratified according to the clinical manifestations of the disease. The frequency of TC/ CC (36.1%) was found to be significantly higher in patients with oral ulcer as compared with patients having TT (7.1%) genotypes (*P*= 0.034). Similar analyses were performed for all other clinical features in **table 5**, but the differences were not statically significant. **Table 6** shows the association of IL-10 (592 C/A) polymorphisms with clinical manifestations in SLE patients. There was no significant association of genotypic distribution with clinical manifestations in SLE patients.

Table 3: Comparison of allele and genotype frequencies of IL-10(-1082G/A), IL-10(-592 C/A) and IL-10(- 819T/C) in SLE patients and controls (n=150)

Genotype and allele	Case (n=75)	Control (n=75)	OR (95% CI)	P-value
IL-10 1082 G/A				
GG	16(21.3%)	12(16.0%)	1.42(0.62-3.26)	0.409
GA	18(24.0%)	19(25.3%)	0.93(0.44-1.96)	0.849
AA	41(54.7%)	44(58.7%)	0.85(0.45-1.62)	0.621
GA/AA	59(78.7%)	63(84.0%)	0.72(0.31-1.61)	0.402
A allele	100(66.7%)	107(71.3%)	0.80(0.49-1.31)	0.382
G allele	50(33.3%)	43(28.7%)		
IL-10 592 C/A				
CC	18(24.0%)	37(49.3%)	0.32(0.16-0.65)	0.001*
CA	41(54.7%)	31(41.3%)	1.71(0.89-3.26)	0.102
AA	16(21.3%)	7(9.3%)	2.43(1.01-6.84)	0.041*
CA/AA	57(76.0%)	38(50.7%)	3.08(1.54-6.19)	0.001*
A allele	73 (48.7%)	45 (30.0%)	2.21(1.38-3.55)	0.001*
C allele	77 (51.3%)	105 (70.0%)		
IL-10 819 T/C				
TT	14(18.7%)	25(33.3%)	0.46(0.22-0.98)	0.041*
TC	36(48.0%)	35(46.7%)	1.05(0.56-2.00)	0.87
CC	25(33.3%)	15(20.0%)	2.00(0.95-4.20)	0.065
TC/CC	61(81.3%)	50(66.7%)	2.17(1.03-4.63)	0.041*
C allele	86(57.3%)	65(43.3%)	1.76(1.11-2.78)	0.015*
T allele	64(42.7%)	85(56.7%)		

Note: *significant
P value reached from Chi-square test

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Table 4: Association of IL-10 (1082 G/A) polymorphisms with clinical manifestations in SLE patients (n=75)

Clinical manifestations	Genotype GA/AA (n=59)	Genotype GG (n=16)	OR (95% CI)	p-value
Rash	34(57.6%)	5(31.3%)	2.99(0.93-9.70)	0.061
Oral ulcer	19(32.2%)	4(25.0%)	1.43(0.41-5.01)	0.579
Arthralgia	38(64.4%)	13(81.3%)	0.42(0.11-1.63)	0.2
Lupus nephritis	20(33.9%)	5(31.3%)	1.13(0.34-3.70)	0.842
Hematological disorder	1(1.7%)	1(6.3%)	0.26(0.02-4.38)	0.316

Note: *significant
P value reached from Chi-square test

Table 5: Association of IL-10 (-819 T/C) polymorphisms with clinical manifestations in SLE patients (n=75)

Clinical manifestations	Genotype TC/CC (n=61)	Genotype TT (n=14)	OR (95% CI)	p-value
Rash	33(54.1%)	6(42.9%)	1.57(0.49-5.07)	0.448
Oral ulcer	22(36.1%)	1(7.1%)	7.33(0.90-59.9)	0.034*
Arthralgia	39(63.9%)	12(85.7%)	0.30(0.06-1.44)	0.115
Lupus nephritis	22(36.1%)	3(21.4%)	2.07(0.52-8.22)	0.295
Hematological disorder	2(3.3%)	0(0.0%)	-	0.492

Note: *significant
P value reached from Chi-square test

Table 6: Association of IL-10 (-592 C/A) polymorphisms with clinical manifestations in SLE patients (n=75)

Clinical manifestations	Genotype CA/AA (n=57)	Genotype CC (n=18)	OR (95% CI)	p-value
Rash	32(56.10%)	7(38.9%)	2.01(0.68-5.94)	0.202
Oral ulcer	20(35.1%)	3(16.7%)	2.70(0.70-10.46)	0.14
Arthralgia	39(68.4%)	12(66.7%)	1.08(0.35-3.35)	0.889
Lupus nephritis	19(33.3%)	6(33.3%)	1.00(0.33-3.08)	1
Hematological disorder	2(3.5%)	0(0.0%)	-	0.421

Note: *significant
P value reached from Chi-square test

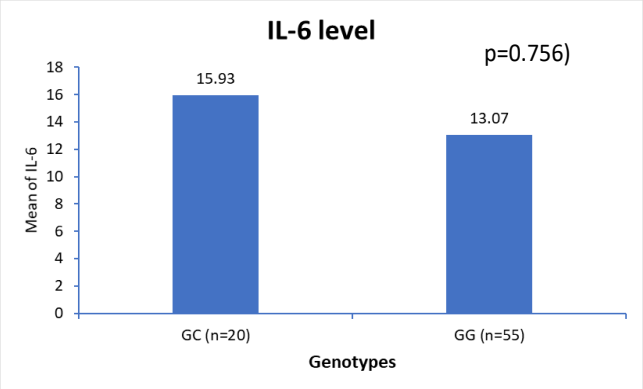

Figure 3: Bar diagram showing IL-6 level according to genotype distribution IL-6 174G/C

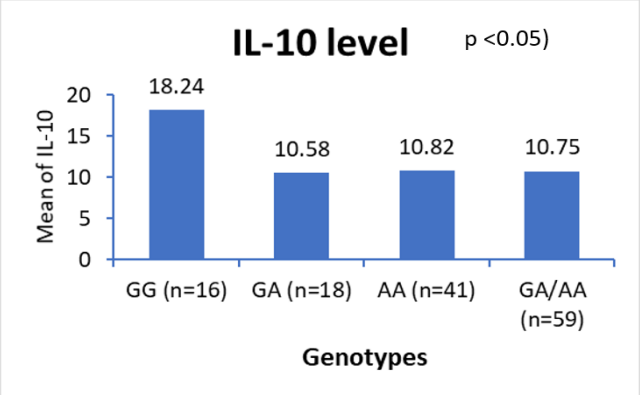

Figure 4a: Bar diagram showing IL-10 level according to Genotypes distribution IL-10 1082G/A

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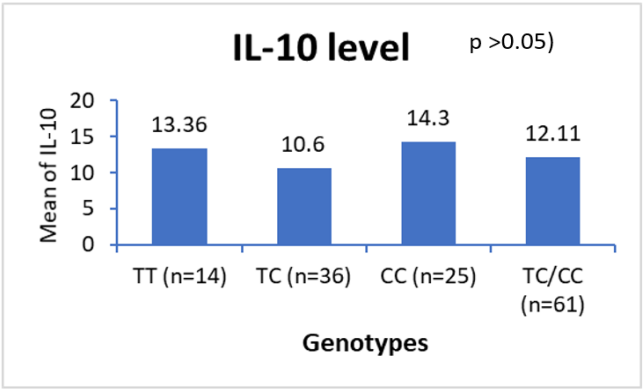

Figure 4b: Bar diagram showing IL-10 level according to Genotypes distribution IL-10 819T/C.

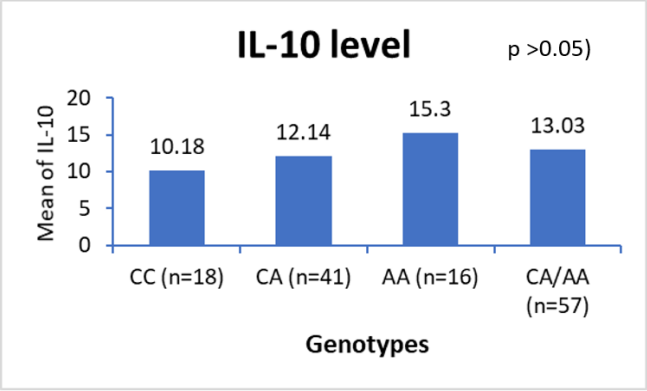

Figure 4c: Bar diagram showing IL-10 level according to Genotypes distribution IL-10 592C/A

Note:

p-value reached from Mann-Whitney U test.

P <0.05 considered as statically significant.

Discussion

SLE is a chronic systemic disease with variable clinical presentation. The exact pathological mechanisms of SLE remains elusive, and the etiology of SLE is known to be multifactorial, involving genes, sex hormones, and environmental factors including sunlight, drugs, and infections. Cytokines have been suggested to be involved in the pathogenesis of SLE, as they are fundamental components in the regulation of immune response, intervening in both cellular and humoral responses. Several immunogenetic studies demonstrated that polymorphisms at several loci, including the major histocompatibility complex, complement proteins, immunoglobulin receptors, cytokine genes and are associated with SLE, implying that the gene products of these loci are involved in the pathogenesis of SLE [24].

The high prevalence of female in this study agreed with a study conducted in Iraq [25], where female represented 98% of total patients involved in that study. The female predominance was reported 88% among 1,103 patients by a largest American study [26]. This finding indicates that female sex is considered as one of the predisposing factors of the disease and the hormones (oestrogen) contribute to increase the prevalence of SLE among women. The gene known to contribute to the pathogenesis of SLE is CD40 which is located on chromosome X [27].

Several genome-wide association studies (GWAS) have confirmed non-HLA genes are linked with SLE. IL-6 is involved in various inflammatory and proliferative diseases, as well as being proposed as a strong candidate for novel targeted biological therapeutics. It has been reported that IL-6 gene polymorphism is associated with susceptibility and outcome of a variety of acute and chronic inflammatory diseases including rheumatoid arthritis, diabetes mellitus, atherosclerosis, Alzheimer disease and juvenile chronic polyarthritis [9]. The earlier studies have identified the polymorphisms in the IL-6 gene locus, which are known to be associated with SLE susceptibility and disease progression [28]. The increased level of IL-6 in SLE patients may derive from constitutively increased cellular production. Peripheral blood mononuclear cell (PBMC) from SLE patients produce significantly more IL-6 upon in vitro stimulation than PBMC from normal control [29]. In the study the homozygous GG genotype of IL-6 (-174G/C) was significantly found in SLE patients compared to healthy controls. The heterozygous GC genotype was found more in healthy controls than SLE patients (26.7%). G allele was found to be significantly higher in cases than control group. Similarly, a study conducted in Malaysia, confirmed the association of GG genotype and G allele of IL-6 (-174G/C) with SLE. In this study it is also suggested that the C allele has a masking effect over the G allele in the heterozygous G/C genotype, which may be due to a complex interaction of both alleles when present co-dominently [30]. In contrast, a study in German Caucasians SLE patients, the IL-6 (-174G/C) polymorphism does not contribute to SLE susceptibility [18]. In this study, any phenotypic association of SLE with the IL-6 (174G/C) polymorphism was not observed. On the other hand, Scotte et al (2001) found that the G allele of IL-6 (-174G/C) is responsible for the presentation of discoid skin leisons in Caucasian German SLE patients [29].

It has been suggested that certain SNPs in the promoter region of IL-10 could be associated with the elevated expression of its cytokine and increased susceptibility to various inflammatory and autoimmune disorders such as asthma, rheumatoid arthritis and SLE [31]. Increased production of IL-10 by peripheral blood mononuclear cells has been associated with-592A allele [32]. In this study the genotypic distribution for the IL-10 (592C/A) gene polymorphism was significantly different between patients

and healthy controls. The homozygous AA genotype was found in higher frequency (21.3%) in SLE patients than (9.3%) healthy control group. The combined genotype CA/AA was significantly found in patients compared to healthy controls. The A allelic frequency was higher (48.7%) in SLE patients compared to control (30.0%). These finding indicates that AA genotype, AA/CA genotype and A allele are associate with the risk of SLE. The finding of this study is similar with another study in India where the-592A allele frequency was significantly higher (46.8%) in SLE patients compared with controls (35.1%) (P=0.0002), and the frequency of CA/AA was also found to be significantly higher (74.5%) in SLE patients than controls (56.2%) (P=0.0001) [33]. Further in a meta-analysis [20] Liu et al. (2013) reported -592C allele to be associated with decreased risk of SLE in an Asian population but not in European population. Similar with this finding, in the present study, IL-10 (-592CC) genotype was increased significantly in controls compared to SLE patients (P=0.001). In the present study there was no significant association of IL-10 (-592C/A) genotype distribution with clinical manifestations. In contrast, The IL-10 (-592 A) allele has been reported to be associated with the development of lupus nephritis among Chinese SLE [21].

This study compared IL-10 (-819T/C) gene polymorphisms with SLE patients and healthy control group. The C allelic frequency was higher (57.3%) in case group than (43.3%) in control group (P=0.015). Moreover, the TT genotype was found in higher frequency in healthy control than patient group(P=0.041). A significant distribution of the -819T/C polymorphism and the C allele are present in Asian SLE patients [22]. Presence of complete linkage disequilibrium between the -819 and -592 alleles have been reported [34]. The function of the -819 C allele in regards to IL-10 production is not completely validated. Increase of the -819 C allele frequency in patients with SLE may not entirely reflect the association of -819 C allele and SLE susceptibility but could be the result of complete linkage disequilibrium between -819 and -592 alleles. In the present study, the frequency of TC/CC (36.1%) was found to be significantly higher in patients with oral ulcer as compared with patients having TT (7.1%) genotype (P=0.034). In the present study, no significant difference in IL-10 (-1082G/A) genotype and allele frequencies was found between control and SLE patients in Bangladeshi population. The association of -1082 G allele with susceptibility to SLE was reported in Caucasians [35]. There was no significant association of IL-10 (-1082G/A) genotype distribution with clinical manifestations in this study. However, Discoid rash in a Spanish [36], Neurological disorders in a Dutch [37] and Renal disorder in a Chinese popuations [21] were found in several studies. And these controversial results about clinical features could be due to the genetic heterogenesity of SLE in different ethnicities.

In this study it was found that patients having IL-10 (-1082 GG) genotype had significantly higher level of serum IL-10 than those with IL-10 (-1082 G/A). No other genotype of IL-6 and IL-10 gene showed such significant association with serum level of cytokines in patient group. The -1082G allele is proven to be the most important positive regulatory factor for the constitutive and inducible expression of IL-10 but the significance of -1082 GG to SLE susceptibility may be diminished by the rarity of -1082G in Asians [38]. In a study, it was found that GG genotype of IL-10 (-1082G/A) associated with higher level of IL-10 but it was not significant due to rarity of G allele [23]. A study in Mexico conducted by Palafox-Sanchez et al (2015) found that patients having CC genotype showed higher IL-10 level than AA genotype of IL-10 (-592C/A) [39]. In contrast, a study in Egypt found no such association with the level of cytokine and genotype distribution of IL-6 and IL-10 gene [2]. These conflicting results may be due to cytokine secretion not only depends on the constitutive production but also inducible factors such as level of other cytokine, ethnicity and age of patients, stimulating agents and cell types etc are associated with plasma level of cytokines. Multi-centered study should be conducted on other SNPs of IL-6 and IL-10 along with other cytokine gene polymorphisms with SLE susceptibility and clinical features of SLE in Bangladeshi population.

Conclusion

The study indicates that GG genotype and G allele of IL-6 (-174G/C) may be associated with susceptibility to SLE while GC genotype of IL-6 (-174G/C) has been associated with decreased susceptibility to SLE in Bangladeshi population. AA genotype, CA/AA genotype and A allele of IL-10 (-592C/A) gene may be associated with the susceptibility to SLE. Moreover, TC/CC genotype and T allelic frequency of IL-10 (-819T/C) gene may also play role in SLE susceptibility. On the contrary, CC genotype of IL-10 (-592C/A) gene and TT genotype of IL-10 (-819T/C) gene are associated with the decreased susceptibility of SLE in Bangladeshi population. The TC/CC genotype of IL-10 (-819 T/C) is associated with oral ulcer of SLE. GG genotype of IL-10 (-1082G/A) gene might influence increased production of IL-10 in SLE patients.

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Authors' contributions

Conception: NN and SA.

Methodology: NN, SA, SKS, RRK and REB.

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Sample collection and laboratory analysis: NN.

Statistical analysis: NN and SA.

Writing—first draft: NN.

Writing—revision and editing: NN and REB.

Supervision: SA, SKS and RRK.

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Declarations Competing interests:

The authors declare that they have no competing interests.

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