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ICOS Gene Polymorphism and Serum Soluble ICOS Level as Genetic and Serological Correlates of Disease susceptibility in Iraqi Patient with Systemic Lupus Erythematosus

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ABSTRACT: **Background**: Systemic lupus erythematosus (SLE) is multifactorial disease arising from breakdown of peripheral immune tolerance, culminating in pathogenic autoantibody production and immune-complex mediate tissue injury. Inducible T-cell Co-Stimulator (ICOS, CD28), a CD28-family co-stimulatory receptor expressed predominantly on activated and follicular helper T (Tfh) cells is central to T-cell B-cell collaboration within germinal centers and has been implicated in the excessive humoral immunity characteristic of SLE. Whether genetic variation at the ICOS locus translates into altered circulating ICOS protein and, in turn, into susceptibility to and severity of SLE remains incompletely defined, particularly in middle-eastern population.

Methods/Objectives: This case-control study aimed to quantify serum soluble ICOS by ELISA and to charcharis ICOS exon-5 single nucleotide polymorphism (rs78281070, rs10172036, rs10183087) by sanger sequencing in Iraqi SLE patient and matched healthy control, and to evaluate the relationship between genotype, serum sICOS concentration and clinical/serological indices of disease.

Result/Discussion: Serum sICOS was markedly elevated in patient relative to control (202.8 ± 10.4 vs 88.9 ± 1.8 pg/mL; P = 0.0001) and discriminated the two groups with high accuracy (AUC=0.92). sICOS correlated inversely with anti-dsDNA, Consistent with a state of net T-cell co-stimulator hyperactivation with impaired counter-regulation. Although individual SNP genotype and allele frequencies did not differ significantly between groups, haplotypes analysis identified a risk associated ATT Haplotype (OR = 2.47, P = 0.047) and protective AGT haplotype (OR = 0.23, P = 0.015), with disease-restricted deviation from Hardy-Wennberg equilibrium at two loci, suggesting that ICOS locus haplotype architecture, rather than any single variant, shapes the serological.

Study Limitation: due to high the high cost associated with sanger sequencing, Genotyping of the ICOS gene polymorphism was restricted to a subset of 40 participant (20 SLE patient and 20 healthy controls

Conclusion: Serum sICOS behaves as a robust, disease-intrinsic biomarker of IOCS/ICOS-L pathway hyperactivation in SLE and show excellent discriminative capacity, while ICOS haplotype architecture offers a plausible though preliminary, genetic substrate for this phenotype. Together, this finding positions the ICOS gene and its

soluble protein products as a dual genetic-serological axis with diagnostic and potential therapeutic relevance in lupus, warranting validation in larger, ethnically matched cohorts.

INTRODUCTION

Systemic lupus erythematosus is an autoimmune inflammatory disease characterized by the presence of flare of autoantibodies, especially against nuclear components. Although it is believed that the etiology of SLE is multifactorial, including immune dysfunction, genetic, hormonal and environmental, the molecular mechanisms underlying this systemic autoimmune response remain largely unknown (Haj afendi & Al Sahlanee, 2025) . Autoantibodies play a central role in the pathogenesis of systemic lupus erythematosus (SLE). The formation and deposition of immune complexes in blood vessels contribute to inflammation and organ damage, particularly in the kidney, brain, and skin. In addition, autoantibody-mediated mechanisms directly cause hematological manifestations such as autoimmune hemolytic anemia and thrombocytopenia through immune-mediated destruction of erythrocytes and platelets (Yuce Inel, Uslu, Demirci Yildirim, Gulle, & Sen, 2025). Disease manifestations can range from mild, self-limiting symptoms to severe, life-threatening organ involvement, this wide variability poses a significant diagnostic challenge, as patients often present with diverse and overlapping clinical features. From a serological perspective, systemic lupus erythematosus (SLE) is defined by the presence of disease-specific autoantibodies, which are crucial for both diagnosis and disease monitoring. About 50–70% of patients have anti-double-stranded DNA (anti-dsDNA) antibodies, which are highly specific for SLE and frequently correlate with disease activity, particularly lupus nephritis (Xagas, Drouzas, Liapis, & Lionaki, 2024). In contrast, anti-Smith (anti-Sm) antibodies are also highly specific for SLE but are less sensitive, appearing in only 20–30% of patients. While anti-Sm antibodies generally do not correlate with disease activity, their presence strongly supports the diagnosis, especially in ANA-positive patients who lack other SLE-specific antibodies (Saleem et al., 2024). Current therapeutic strategies in systemic lupus erythematosus (SLE) aim to achieve and maintain remission, prevent irreversible organ damage, and improve long-term quality of life and survival outcomes. Advances in disease management have led to significant improvements in prognosis, including better survival outcomes for patients with severe organ involvement such as lupus nephritis (Desai, Gashti, & Whittier, 2026). Costimulatory receptors such as ICOS (Inducible T-cell Co-Stimulator) They function as activating signals that enhance T-cell responses, promoting full immune activation rather than inhibition. This positive regulation is essential for effective immune defense, supporting T-cell proliferation, survival, and T-cell–B-cell interactions, which are crucial for antibody production and adaptive immune responses (Houssaini et al., 2023b). When costimulatory pathways such as ICOS signaling are dysregulated, the immune system can become overactivated. In diseases like systemic lupus erythematosus (SLE), enhanced ICOS-mediated T-cell help promotes excessive T-cell activation and abnormal T–B cell interactions, leading to increased autoantibody production. These autoantibodies contribute to the attack on healthy tissues, resulting in chronic inflammation and multi-organ damage (J. Qi, C. Liu, Z. Bai, X. Li, & G. Yao, 2023). Inducible T-cell Co-Stimulator (ICOS) is a protein expressed on the surface of activated T cells, particularly follicular helper T (Tfh) cells. It plays a crucial role in regulating immune responses by interacting with its ligand (ICOSL) on antigen-presenting cells, such as dendritic cells and B cells, thereby enhancing T-cell activation and supporting T–B cell interactions. Through this pathway, ICOS promotes cytokine production, germinal center formation, and antibody generation. Dysregulation of ICOS signaling has been associated with autoimmune diseases, including systemic lupus erythematosus (SLE), where it contributes to excessive immune activation and autoantibody production, suggesting its potential as a therapeutic target. The current study hypothesized to investigate the serum level of soluble ICOS (sICOS) in patients with SLE (Kim et al., 2024), as well as correlating these levels with biological indices of patients.

2. MATERIALS AND METHODS

Study design and sampling

This case- control study aimed to investigate the effect of ICOS gene polymorphisms on the expression level of this gene and their association with the Iraqi population. A total of 112 participants were recruited from the Department of Rheumatology, Baghdad Teaching Hospital, Medical City in Baghdad, Iraq. Their demographic, clinical and serological data are listed in Table 1. All patients satisfied the American College of Rheumatology (ACR) 1997, and SLICC 2012 classification criteria (Petri et al., 2012),(Hochberg, 1997) and met at least four of the criteria for SLE. The diagnosis of SLE was based on the clinical and immunologic criteria: malar rash (MR), photosensitivity (PS), discoid rash, oral or nasal ulcers, arthritis, neurologic disorder, hematologic disorder (hemolytic anemia, leukopenia or lymphopenia and thrombocytopenia) and the presence of anti-nuclear Abs, such as Abs anti-dsDNA, anti-Sm. Complement levels (C3, C4) represented the consumption of early components of the conventional complement pathway as a result of immunocomplexes deposition in tissues. Healthy controls were selected to be

comparable to the patient group in terms of age and gender. They were clinically asymptomatic and showed no evidence of autoimmune or inflammatory disorders based on clinical evaluation and routine laboratory investigations.

Ethical compliance

All samples were collected following informed consent and in accordance with ethical standards approved by The Medical City Hospital Ethics Committee and the University of Baghdad's Committee for Postgraduate Studies (CSEC/0526/0047).

Exclusion criteria

Our study cohort excluded patients with any SLE-allied disease and other unspecified conditions (liver disease, thyroid and/or parathyroid disorders, diabetes mellitus, fibromyalgia, malignancies, pregnancy, neuropsychiatric SLE and any other inflammatory diseases or overlap syndromes except for secondary antiphospholipid syndrome and primary Sjogren syndrome [pSS]).

Sample collection

A total of 112 blood samples were collected from SLE patients and healthy control groups from the period of November 2025 to january2026. All samples were divided into two parts 2mL of EDTA tubes and 2mL in the gel tube. Next to, all the samples were subjected to the following tests: CBC, ESR, anti-double strand DNA, anti-smith (using ELISA kits), and the serum soluble ICOS (sICOS) level through ELISA kits. Two mL of blood was centrifuged for 5 min at 1107g for serum collection. All samples were kept at -20 °C for further analysis.

Serum ICOS Analysis

ICOS (Inducible T-cell Co-Stimulator) serum concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ELK Biotechnology, catalog number ELK3134), according to the manufacturer's instructions. Briefly, standards, serum samples, and controls were added to microplate wells pre-coated with a monoclonal antibody specific for human ICOS. The immobilized antibody captured ICOS present in the samples. After washing to remove unbound material, an enzyme-linked polyclonal antibody specific for ICOS was added. Following further washing steps, a substrate solution was introduced, resulting in color development proportional to the amount of ICOS bound in the initial step. The reaction was terminated by the addition of a stop solution, and absorbance was measured at 450 nm using a microplate reader. Serum ICOS concentrations were calculated from the standard curve and expressed in pg/mL.

SNP selection and genotyping

The selection of polymorphisms in the *ICOS* gene (NG_011586.1) was based on their reported location within exon 5 of the gene, their proximity to one another (allowing all variants to be captured within a single amplicon), and their reported association with autoimmune disease susceptibility AID in the literature (Fengyan Xu et al., 2011). Thus, three polymorphisms in the ICOS gene were selected: rs78281070 (+ 519 A/C), rs10172036 (+435 T/G) and rs10183087 (+394 T/C). The extraction of genomic DNA from peripheral blood leucocytes of each subject was performed by the standard procedure of EasyPure® Genomic DNA Kit (TransGen, biotech. EE101-01).

A single pair of primers (Forward: 5'-TGCAATCAGAGGTGGACAGT-3' ; Reverse: 5'-

GTGAAATGCGGACAGATGCT -3'; designed with Primer3, https://primer3.ut.ee/) was used to amplify a 695 bp fragment spanning exon 5 of ICOS (chr2:203,959,108,203,960,092,GRCh38.p14) , encompassing all three SNPs (rs78281070 , rs10172036 and rs10183087). PCR amplification was carried out in a final volume of 25 µL reaction containing 12.5 µL 2x EasyTaq® PCR SuperMix (TransGen Biotech, AS111-01), 1 µL each of forward and reverse primer (10 µM), 5 µL template DNA, and 4.5 µL nuclease -free water. Four annealing temperatures (56, 58, 60, and 62°C) were tested, and 58°C was selected as optimal. Cycling conditions were: initial denaturation at 94°Cfor 5 min; 35 cycles of 94°C for 30s, 58°C for 30s, and 72°C for 30s; and a final extension at 72°C for 5min. PCR products were verified by electrophoresis on a 2% (w/v) agarose gel.

Sanger sequencing

Purified PCR products were sequenced directly (dideoxy chain termination technique) on an ABI3730XL automated DNA sequencer (Macrogen Corporation, Korea). Genotyping was performed on a subset of 20 SLE patients and 20 healthy controls, selected at random from the full cohort of 56 patients and 56 controls, owing to the cost of Sanger sequencing relative to the full sample. Chromatograms were aligned to the ICOS reference sequence (GenBank AH011568.2) and genotypes were called from

the resulting contig assemblies.

Statistical analysis

Data analysis was done by utilizing SPSS for Windows, version 25 (SPSS Inc. Chicago, Illinois, United States), and analysis with graphing were performed using Graph-Pad Prism version 8 (San Diego, CA, USA). Categorical variables were expressed as frequencies and proportions. Proportions were compared using the Chi-square test (χ^2). Data were expressed as mean ± standard error (SE) for continuous variables. Statistically difference between groups were analysed by T-tests or one way ANOVA tests. The ROC (Receiver Operating Characteristic) curves would facilitate the evaluation of the diagnostic ability of the tests variables by calculating the area under the curve (AUC). A P value less than 0.05 were considered statistically significant and P value less than 0.01 considered highly statistically significant (Siepsiak, Vrana, Rynkiewicz, Rosenthal, & Dragan, 2022).

3. RESULT:

Demographic characteristic of the study population

A total of 112 subjects were enrolled in this case-control study, comprising 56 patients fulfilling the ACR-1997/SLICC-2012 classification criteria for systemic lupus erythematous and 56 apparently healthy, age and sex matched, controls. Patients ages range from 17 to 68 years (mean = 38.59 ± 11.81 years), while controls ranged from 19 to 53 years (mean = 39.54±10.71 years); the two groups did not differ significantly in age distribution (χ^2 = 4.9, P = 0.28), confirming adequate demographic matching (Table 1). The disease predominated in the fourth and fifth decades of life (31-40 and 41-50 years, 26.8% each), consistent with the recognized tendency of SLE to affect women of reproductive and peri-reproductive age (Tian, Zhang, Yao, Huang, & Lu, 2023).

Females constituted 85.7% of the patient group compared with 14.3% males, corresponding to a female-to a male ratio off approximately 6:1; this ratio, although markedly skewed, did not differ signifyingly from the six distributions of the control group (x^2 =0.97, P = 0.32). The observed predominate lies within the 4:1-11:1 range reported internationally (Albrecht et al., 2025)and is compatible with the even more pronounced female preponderance (95-97%) describe in Iraqi cohorts (Hashim et al., 2025), reinforcing the well-established contribution of X-linked and hormonal factors to lupus susceptibility (Scofield & Lewis, 2024).

Table 1: Demographic distribution of SLE patients and healthy controls.

Variable	Category	Patients n (%)	Controls n (%)	χ^2	P-value
Age (years)	≤20	4 (7.1%)	5 (8.9%)	4.9	0.28 NS
	21–30	13 (23.2%)	23 (41.1%)		
	31–40	15 (26.8%)	11 (19.6%)		
	41–50	15 (26.8%)	12 (21.4%)		
	>50	9 (16.1%)	5 (8.9%)		
Sex	Male	8 (14.3%)	12 (21.4%)	0.97	0.32 NS
	Female	48 (85.7%)	44 (78.6%)		

NS: not significant (P > 0.05).

Hematological and serological profile

Compared with controls, SLE patient showed comparable red blood cell (RBC) count (4.4 ±0.12vs4.7±0.09×10^{12}/L; P = 0.82), but a significantly white blood cell (WBC) count (7.6 ± 0.4 vs 6.9 ± 0.25 ×10^9/L; P = 0.003) and markedly elevated erythrocyte sedimentation rate (ESR) (30.09 ± 2.8 vs 14 ± 1 mm/h; P < 0.0001), reflecting the systemic inflammatory burden of active disease,

although corticosteroid induced neutrophilia may also have contribute to the WBC elevation (Cabrera & Castiblanque, 2025),(Dima, Opris, Jurcut, & Baicus, 2016). Serologically, both anti-dsDNA (3.23 ± 0.15 vs 2.36 ± 0.07; P = 0.006) and anti - Sm antibody titers (0.39 ± 0.01 vs 0.16 ± 0.002; P = 0.0001) were significantly higher in patient, consistent with their established role as lupus-specific autoantibody ((Xagas et al., 2024),(Saleem et al., 2024). The most frequent clinical manifestation among patient were arthritis (76.8%), malar rash (44.6%) and photosensitivity (35.7%), as show in Table 2.

Table 2:

Hematological profile analysis Mean ± SE			
	Patient	Control	*P* value
RBC	4.4±0.12	4.7±0.09	0.82^{NS}
WBC	7.6±0.4	6.9±0.25	0.003**
ESR	30.09±2.8	14±1	<0.0001**
Data present as T- test, ** highly significantly (p<0.01), NS (non-significant).			
immunological landscapes of SLE patients			
Anti-Sm	0.39±0.01	0.16±0.002	0.0001**
Anti-dsDna	3.23±0.15	2.36±0.07	0.006**
Data present as T- test, ** highly significantly (p<0.01), NS (non-significant).			

Clinical manifestation of SLE (n)%		
Arthritis	43/56	(76.8%)
Malar rash	25/56	(44.6%)
Photo sensitivity	20/56	(35.7%)

Serum Soluble ICOS (sICOS) levels: in patient and controls

Serum sICOS concertation, measured by ELISA, was markedly and highly significant elevated in SLE patient relative to healthy controls (202.8 ± 10.4 pg/mL vs 88.9 ± 1.8 pg/mL; P = 0.0001), representing in approximately 2.3-fold increase (Table 3). This finding provides direct serological evidence of ICOS pathway hyperactivation in lupus and is concordant with cell- surface flow-cytometric data showing expanded ICOS -expressing $CD4^+$ and $CD8^+$ T-cell population in SLE (Yang et al., 2005) and with functional evidence that ICOS co-stimulation potentialities IgG anti-dsDNA production by B cells from patient with active disease (M. Kawamoto et al., 2006).

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Table 3: Mean serum sICOS concentration in SLE patients and healthy controls.

Group	Mean ± SE (pg/mL)	*P-value*
SLE patients (n = 56)	202.8 ± 10.4	0.0001 **
Healthy controls (n = 56)	88.9 ± 1.8	

highly significant (P < 0.01); data analyzed by independent-samples t-test.

Diagnostic performance of serum sICOS (ROC Curve Analysis)

Receiver operating characteristic (ROC) analysis (Wilson/brown method) showed that serum sICOS discriminated SLE patient from healthy controls with very good accuracy, yielding an Aerea under the curve (AUC) of 0.92.92 ± 0.027 (95% CI: 0.8717–0.9778; P < 0.0001). At the optimal cut-off of 112.9pg/ml. sICOS achieved sensitivity 98.2%, specify only 85.7%) and approaching, though not matching, the near perfect discrimination of anti-sm (AUC = 0.99) measured in parallel within the same cohort.

Table 4: ROC curve indices for serum biomarkers distinguishing SLE patients from controls.

Marker	Sensitivity (%)	Specificity (%)	AUC ± SE	95% CI	Cut-off	P-value
sICOS	98.2	85.7	0.92 ± 0.027	0.8717–0.9778	112.9 pg/mL	<0.0001
anti-Sm	98.2	100	0.99 ± 0.001	0.9952–1.000	0.21	<0.0001
anti-dsDNA	100	25	0.78 ± 0.043	0.7005–0.8690	3.6	<0.0001

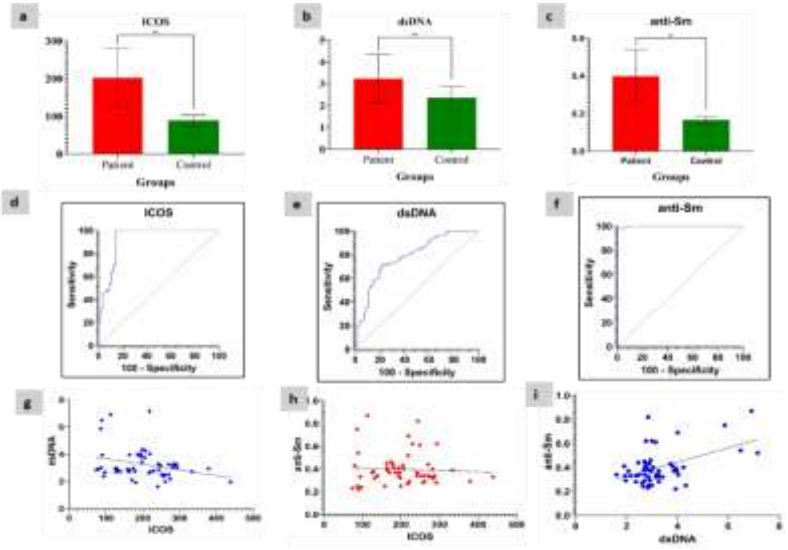


Figure (1): Serum levels and ROC curve indices for serum biomarkers distinguishing SLE patients from controls. A, B and C: serum level of ICOS, dsDNA and anti-Sm, respectively, in patients and healthy controls. D, E and F: Roc curve analysis of ICOS, dsDNA and anti-Sm, respectively. G: correlation coefficient between ICOS and dsDNA. H: correlation coefficient between ICOS and anti-Sm. I: correlation coefficient between dsDNA and anti-Sm.

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Correlation of serum sICOS with disease -associated biomarkers

Within the patient group, Pearson correlation analysis revealed a significant relationship between sICOS and anti-dsDNA (r = −0.289, P = 0.031) (TABLE) no significant correlation was detected between sicos and anti-Sm titers (r = −0.058, P = 0.671). In contrast, anti-dsDNA correlated positively with anti-Sm (r = 0.477, P < 0.001) (Table 5).

Table 5: Correlation matrix among serum, sICOS, anti-dsDNA and anti-Sm in SLE patients.

	sICOS (r / P)	**anti-dsDNA (r / *P-value*)**	**anti-Sm (r / *P-value*)**
sICOS	1	−0.289 / 0.031 *	−0.058 / 0.671 NS
anti-dsDNA	−0.289 / 0.031 *	1	0.477 / <0.001 **
anti-Sm	−0.058 / 0.671 NS	0.477 / <0.001 **	1

Significant (P < 0.05); ** highly significant (P < 0.01); NS: non-significant.

Serum sICOS in relation to clinical and Therapeutic Subgroups

Figure (2) presented no significant different in serum sICOS were detected according to disease duration (≥5 vs. <5 years: 196.5 ± 12 vs 217.4 ± 20.6 pg/mL, P = 0.36), treatment status (treated vs. untreated: 207.4 ± 11.7 vs 183.8 ± 22.6 pg/mL, P = 0.37), the specific immunosuppressive/DMARD regimen revived (one-way ANOVA, P = 0.8). The absence of a treatment- related gradient suggests that circulating sICOS reflects a relative stable, disease-intrinsic feature of the ICOS/ICOS-L axis rather than a therapy-modifiable epiphenomenon, at least across the heterogenous, partially treated cohort studied here.

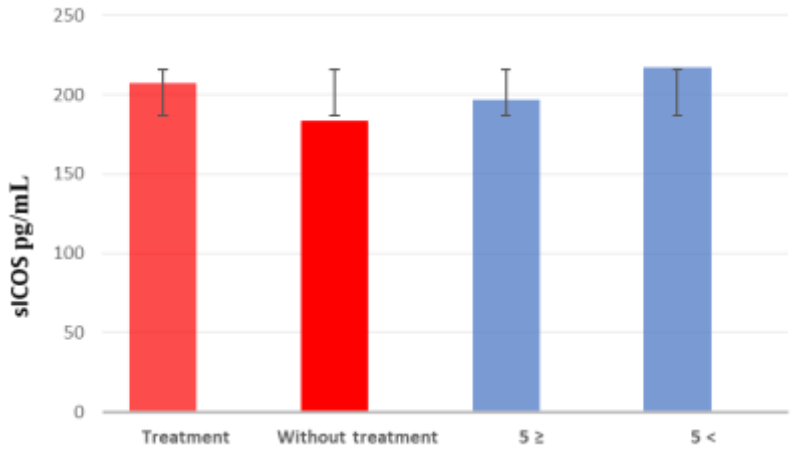


Figure (2): Serum sICOS in relation to clinical and Therapeutic Subgroups. Red column showed treatment status (treated vs untreated) and the blue columns represent disease duration, all subgroups showed non-significant different.

Gel electrophoresis result

The figure below shows the gel electrophoresis result of the *ICOS* gene (695 bp). The presence of the expected bands indicates successful amplification of the targeted gene.

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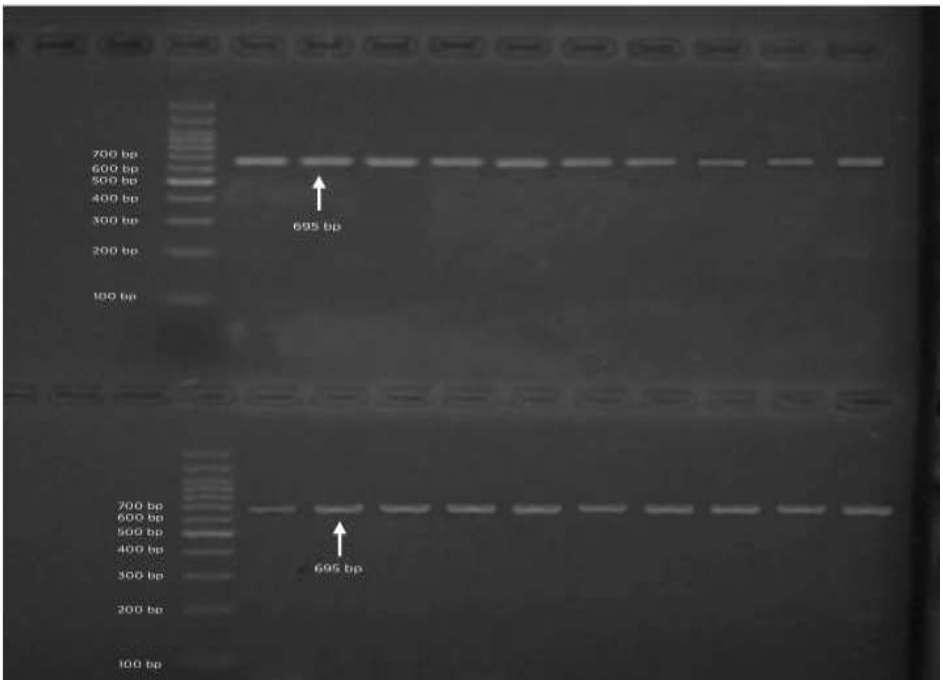


Figure (3): gel electrophoresis of *ICOS* gene (695 bp). The bands in the upper part of the figure represent the *ICOS* gene in the samples of controls. The bands in the lower part of the figure show the *ICOS* gene in patient samples.

ICOS gene polymorphisms: Genotype and allele distribution

Genotyping of the ICOS exon-5 amplicon by sanger sequencing was performed in randomly selected subset of 20 SLE patient and 20 controls. Of the polymorphism examined, rs78281070 (+519 A>C) , rs10172036 (+435 T>G) and rs10183087 (+394 T>C) were all polymorphic (Table 6).

Table 6: Genotype and allele frequencies of thee polymorphic ICOS SNPs in SLE patient and controls.

SNP	Genotype/Allele	Patients n (%)	Controls n (%)	OR (95% CI)	P-value
rs78281070 (A>C)	AA	12 (60.0)	14 (70.0)	Reference	—
	AC	6 (30.0)	6 (30.0)	1.17 (0.24–5.69)	1.000 NS
	CC	2 (10.0)	0 (0.0)	5.80 (0.25–132.57)	0.482 NS
	Allele A	30 (75.0)	34 (85.0)	Reference	—
	Allele C	10 (25.0)	6 (15.0)	1.89 (0.54–7.08)	0.264 NS
rs10172036 (T>G)	TT	10 (50.0)	6 (30.0)	Reference	—
	TG	4 (20.0)	6 (30.0)	0.40 (0.08–2.02)	0.422 NS
	GG	6 (30.0)	8 (40.0)	0.45 (0.10–1.95)	0.464 NS
	Allele T	24 (60.0)	18 (45.0)	Reference	—
	Allele G	16 (40.0)	22 (55.0)	0.55 (0.22–1.33)	0.179 NS

SNP	Genotype/Allele	Patients n (%)	Controls n (%)	OR (95% CI)	P-value
rs10183087 (T>C)	TT	14 (70.0)	10 (50.0)	Reference	—
	TC	2 (10.0)	8 (40.0)	0.18 (0.03–1.03)	0.063 NS
	CC	4 (20.0)	2 (10.0)	1.43 (0.22–9.38)	1.000 NS
	Allele T	30 (75.0)	28 (70.0)	Reference	—
	Allele C	10 (25.0)	12 (30.0)	0.78 (0.29–2.08)	0.617 NS

None of the three SNPs reached statistical significance for individual genotype or allele association with SLE susceptibility at this sample size, although two trends deserve note: the C allele of rs78281070 was numerically 1.9-fold more frequent in patient (25.0% vs. 15.0%) and the TC heterozygote of rs10183087 was under-represented in patient relative to controls (10.0% vs. 49.0%, P = 0.063), approaching but not reaching conventional significance.

Hardy -Wenberg Equilibrium (HWE)

Genotype distribution in the control group conformed to Hardy-Weinberg expectation for all three SNPs (P > 0.05). in the patient group, however, rs10172036(χ^2 = 6.806, P = 0.009) and rs10183087(χ^2 = 10.756, P = 0.0010) deviated significantly from HWE deviation confined to case is often interpreted as a marker of disease-associated selection at, or genetically linked to that locus although at n = 20 per group this finding must be regarded as hypothesis-generating and warrants confirmation in a large, independently genotype cohort before firm interference of biological selection can be drawn.

Table 7: Hardy–Weinberg equilibrium testing for each polymorphic ICOS SNP.

SNP	Cases (χ^2, P)	Controls (χ^2, P)
rs78281070	0.800, 0.371 NS	0.623, 0.430 NS
rs10172036	6.806, 0.009 **	3.104, 0.078 NS
rs10183087	10.756, 0.0010 **	0.045, 0.831 NS

Haplotype / Linkage Disequilibrium (LD) Analysis

Because all three SNPs lie within a 695 bp amplicon spanning exon 5 of ICOS, pairwise linkage disequilibrium (LD) was assessed by the expectation-maxmaiton algorithm. rs78281070andrs10172036 were in complete LD (|D'| = 1.00) in both patient and controls, although the correlation coefficient (r^2) was markedly higher in patient (r^2 = 0.50) than controls (r^2 = 0.14). The rs10172036- rs10183087 pair showed strong LD in patient (D' = 1.00, r^2 = 0.50) but weaker LD in controls (D' = 0.57, r^2 = 0.11); rs78281070-rs10183087 showed moderately strong LD in both groups (D' = −0.73 to −1.00) (Table 8).

Table 8: Pairwise D' and r^2 among the three polymorphic ICOS SNPs, by group.

SNP pair	D' (patients)	r^2 (patients)	D' (controls)	r^2 (controls)
rs78281070–rs10172036	−1.00	0.50	−1.00	0.14
rs78281070–rs10183087	−0.73	0.53	−1.00	0.41
rs10172036–rs10183087	1.00	0.50	0.57	0.11

Haplotype reconstruction of the three-locus (rs78281070-rs10172036-rs10183087) block identified six haplotypes with frequency ≥3% (Table 9). Strikingly, the ATT haplotype was significant over-represented in patient relative to control (60.0% vs. 37.8%; OR = 2.47, 95% CI: 1.004–6.067; P = 0.047) indicating a normally significant risk effect, whereas the AGT haplotype was significant under-represented in patient (10.0% vs. 32.2%; OR = 0.23, 95% CI: 0.069–0.798; P = 0.015), suggesting a protective association. the remaining haplotypes (AGC, CGC, CGT, CTC) did not differ significant between groups.

Table 9: Estimated haplotype frequencies of ICOS SNPs (rs78281070–rs10172036–rs10183087) in patients and controls.

Haplotype	Cases, freq.	Controls, freq.	OR (95% CI)	P-value
ATT	24 (0.600)	15 (0.378)	2.47 (1.004–6.067)	0.047 *
AGT	4 (0.100)	13 (0.322)	0.23 (0.069–0.798)	0.015 *
AGC	2 (0.050)	6 (0.150)	0.30 (0.056–1.578)	0.136 NS
CGC	8 (0.200)	3 (0.078)	2.96 (0.735–11.883)	0.115 NS
CGT	2 (0.050)	0 (≈0.000)	—	0.152 NS
CTC	0 (≈0.000)	3 (0.072)	—	0.084 NS

Haplotypes with frequency <0.03 were excluded from analysis. * Significant (P ≤ 0.05); NS: non-significant.

4. DISCUSSION

Serum sICOS as a serological signature of ICOS/ICOS-L pathway hyperactivation

The central finding of this study- a highly significant, more than two-fold elevation of serum sICOS in SLE patients relative to healthy controls, coupled with excellent ROC discrimination (AUC = 0.92), provides strong serological corroboration of a mechanism long inferred from cell-surface and functional immunology: chronic overactivation of the ICOS/ICOS-L co-stimulatory axis in lupus. ICOS is not constitutively expressed on resting T lymphocytes; rather, its surface expression is induced upon T-cell receptor engagement and amplified by CD28 co-stimulation, after which ICOS ligation by ICOS-L on antigen-presenting and follicular dendritic cells, as well as on B cells, drives PI3K/Akt-dependent intracellular signaling that sustains T-cell survival, promotes secretion of IL-10 and IL-21, and is indispensable for the acquisition of the follicular helper T (Tfh) phenotype (Jingjing Qi, Chang Liu, Ziran Bai, Xia Li, & Genhong Yao, 2023),(Houssaini et al., 2023a). In the germinal center, ICOS-ICOS-L interaction between Tfh cells and cognate B cell licenses somatic hypermutation, class-switch recombination and the selection of high-affinity B-cell clones; when this checkpoint is dysregulated, as occurs in SLE, the same machinery that normally curates protective antibody responses instead licenses autoreactive B-cell clones directed against nuclear antigens (Su et al., 2024).

Soluble ICOS is thought to arise chiefly through proteolytic cleavage (ectodomain shedding) of the membrane-bound receptor and, to a lesser extent, alternative splicing of ICOS transcripts that yield a secretable isoform lacking the transmembrane domain. Because shedding intensity scales with the density of activated, ICOS-high T cells, circulating sICOS can be regarded as a surrogate, integrative readout of total-body ICOS-pathway activity rather than a mere bystander protein. The magnitude of elevation observed here is congruent with flow-cytometric reports of expanded $ICOS^+CD4^+$ and $ICOS^+CD8^+$ T-cell subsets in SLE (Yang et al., 2005) and with functional co-culture data demonstrating that ICOS engagement potentiates IgG anti-dsDNA secretion by B cells form patients with active disease Potentiates IgG anti-dsDNA secretion by B cells from patients with active disease (Manabu Kawamoto et al., 2006). More recent single-cell studies extend this picture further, showing that ICOS-positive circulating Tfh1 and Tfh17 subsets correlate with disease activity and with expansion of activated, extrafollicular-like B-cell populations, and that ICOS-positive Tfh17/Tfh17.1 cells specifically drive anti-dsDNA autoantibody production (T. Khunsri et

al., 2025). Taken together, the serum sICOS elevation documented in the present cohort is best interpreted as the humoral echo of an expanded, hyperactive Tfh/ICOS compartment that continuously licenses autoreactive germinal-centre output.

Genotype-phenotype correlation: from ICOS SNPs to protein expression and autoimmunity

Although none of the three exon-5 SNPs individually reached significance for genotype or single-allele association at this sample size, the multi-locus haplotype analysis revealed a coherent and biologically plausible signal: the ATT haplotype conferred a significance risk (OR = 2.47, P = 0.047), while the AGT haplotype was protective (OR = 0.23, P = 0.015). this pattern illustrates a principle now well established in the immunogenetics of co-stimulatory receptors-that disease risk frequently resides not in any single SNP acting alone, but in specific cis-haplotype combinations that jointly determine transcriptional output, mRNA stability or splicing efficiency of the ICOS locus (Houssaini et al., 2023a),(F. Xu et al., 2011). Exon-5 variants, even when synonymous or non-coding at the amino-acid level, can perturb exonic splicing enhancer/silencer motifs or alter local mRNA secondary structure, thereby modulating the ratio of membrane-bound to soluble ICOS isoforms, transcript half-life, or the efficiency of ribosomal loading-mechanisms directly analogous to those described for non-coding autoimmune-risk variants that reshape ICOS expression in T-peripheral-helper cell development (Kim et al., 2024). The complete linkage disequilibrium between rs78281070 and rs10172036, and the markedly higher r^2 for both this pair and the rs10172036–rs10183087 pair in patients relative to controls, is itself suggestive of disease-associated haplotype restructuring at this locus, consistent with selective pressure acting on a functional variant carried on the ATT background.

The internal consistency between the genetic and serological arms of this study- an ATT-enriched, AGT-depleted haplotype architecture in patients, occurring against a background of markedly elevated serum sICOS- supports a working model in which specific ICOS haplotypes bias transcriptional or post-transcriptional regulation toward increased ICOS expression and/or shedding, which in turn sustains excessive Tfh-B cell crosstalk, germinal-center hyperactivity and autoantibody production. This genotype-to-phenotype trajectory mirrors the broader paradigm established for CD28-family co-stimulatory genes in SLE, in which regulatory polymorphisms translate into quantitative shifts in protein expression that are only detectable when genetic and protein-level data are examined jointly, as performed here.

It should nonetheless be emphasized that the HWE deviation observed for rs10172036 and rs10183087 exclusively within the patient subgroup, together with the wide confidence intervals surrounding all odds ratios (a direct consequence of the n = 20 genotyped patients and controls), substantially limits the precision of these genetic estimates. The haplotype associations, although nominally significant, did not survive explicit correction for multiple comparisons across the six haplotypes tested and must therefore be regarded as preliminary rather than conclusive.

Comparison with previous studies and cross-ethnic variation in allele frequencies

Direct comparison of allele frequencies across populations reinforces the importance of ethnic background in ICOS immunogenetics studies. The C-allele frequency for rs78281070 observed among Iraqi controls in the present study (15.0%) and the corresponding frequency among patients (25.0 %) both differ appreciably from frequencies reported in other case-control series of ICOS polymorphisms and SLE susceptibility, such as the North-African cohort described by (Houssaini et al., 2023a), underscoring that allele frequency at co-stimulatory loci is not uniform across Middle-Eastern, North-African, East-Asian and European populations. Such variation has direct methodological implications: odds ratios computed against a population-specific reference allele frequency are not directly transportable across ethnic groups, and apparent discrepancies between studies reporting significant versus null SNP associations may, at least in part, reflect differences in background haplotype structure and minor-allele frequency rather than true biological heterogeneity in disease mechanism. This consideration argues for cautious, population-matched interpretation of the present findings and for their eventual replication in larger, multi-center Iraqi and regional cohorts before generalization.

With regard to the serum biomarker findings, the magnitude and diagnostic accuracy of sICOS elevation reported here (AUC = 0.92) clearly superior to the moderate discriminative power of anti-dsDNA alone (AUC = 0.78)- a pattern consistent with the increasingly recognized value of soluble co-inhibitory/co-stimulatory checkpoint proteins as complementary, rather than replacement, biomarkers alongside classical lupus serology (McKinski et al., 2022). It is important to note, however, that specifically cautioned that some commercial sICOS ELISA platforms are susceptible to non-specific interference, a methodological limitation that applies equally to the present assay and tempers unqualified acceptance of the observed AUC until cross-validated with an orthogonal quantification method.

The sICOS -dsDNA-anti-Sm correlation network: an immunological interpretation

The weak but significant inverse correlation between sICOS and anti-dsDNA (r = -0.289, P = 0.031) is less immediately intuitive, given the biological expectation that greater ICOS driven Tfh help should, if anything favors higher rather than lower autoantibody titers. Several non-mutually -exclusive explanation merit consideration the cross—sectional design capture a single time -point rather than the temporal sequence of ICOS -pathway activation and downstream antibody production, so that patient sampled during an early or resolving phase of ICOS -driven-cell help might show discordant instantons levels of the two markers sustained (Xu et al., 2017), very high sICOS shedding could reflect receptor down-modulation or exhaustion of the ICOS-high Tfh compartment following an antecedent burst of antibody-producing activity, producing an inverse temporal relationship even under a causally activated model and soluble ICOS measured by ELISA may not fully capture membrane-bound, functionally engaged ICOS on the Tfh cells that are proximally responsible for driving anti-dsDNA producing, so that two assay are, to some degree measuring biologically distinct compartment of the pathway. By constat the robust positive correlation between anti-dsDNA and anti-Sm (r = 0.477, P < 0.001) is readily explained by their shared dependence on Tfh -licensed germinal center output direct against nucleosomal and spliceosomal antigen, and serves as an internal positive control validating the serological assays used (Tipanan Khunsri et al., 2024). The absence of a significant relationship between sICOS and either treatment status, disease duration, RA overlap, or specific immunosuppressive regimen, suggest that circulating sICOS behaves as a relatively trait-like, disease-intrinsic marker within this cohort rather than a short-term, state-dependent index of current pharmacological control—a property that, if confirmed longitudinally, would be advantageous for its candidacy as a susceptibility\trait biomarker rather than purely a flare marker though larger, prospectively sampled cohort with formal SLEDAI scoring and paired C3\C4 measurement are required to properly test its relationship with real-time disease activity and lupus nephritis ,as has been described for sICOS elsewhere in the literature.

Therapeutic implication: the ICOS\ICOS-L Axis as a druggable target

The convergent genetic and serological evidence for ICOS-pathway hyperactivation in this Iraqi SLE cohort lends further support to ongoing effort to therapeutically target the ICOS\ICOS-L axis. Because ICOS engagement is essential for Tfh differentiation, germinal-centra persistence and autoantibody-secreting plasmablast generation, pharmacological blockade of ICOS or ICOS-L whether through neutralizing monoclonal antibodies, ICOS-fc fusion constructs, or antibody-dependent depletion of ICOS-high Tfh cells—has emerged as a rational strategy to interrupt the T-cell-B-cell collaboration that sustains autoreactivity in SLE, complementing established B-cell-depleting and interferon-pathway-targeted biologics discussed in contemporary EULAR management guidance (Fanouriakis, Kostopoulou, Bertsias, & Boumpas, 2024). Within this framework serum sICOS and ICOS-associated haplotype status could, in principle, serve a dual translational purpose: as a pharmacodynamic biomarker to confirm target engagement and pathway suppression during anti-ICOS\ICOS-L therapy dysregulation and who might therefore derive greater benefit increasingly advocated for costimulatory-pathway-directed biologics in lupus nephritis and refractory disease (Desai et al., 2026).

5.CONCLUSION:

This study provides convergent genetic and potential-level evidence that the ICOS/ICOS-L costimulatory axis contributes to the immunopathogenesis of systemic lupus erythematous (SLE). Serum soluble (sICOS) was significantly elevated in patients irrespective of treatment status and showed excellent discriminative accuracy (AUC = 0.92), supporting its role as a stable humoral signature of an expanded, hyperactive T follicular helper (Tfh) compartment, in line with cellular evidence linkage ICOS – expressing Tfh subsets to autoantibody – driven pathology. Genetically, none of the three 5 variants was individually associated with SLE; however, a significant haplotype effect (ATT, risk; AGT, protective) together with patient restricted Hardy-Weinberg deviation suggest that ICOS influence susceptibility primarily through haplotype-block architecture rather than isolated variants. Future adequately powered studies in Middle Eastern populations should therefore favor haplotype -based over single guided trial enrichment, while sICOS represents an ELISA -accessible adjunct to conventional anti-dsDNA and anti-Sm serology. Given the reported efficacy of ICOS-L blockade (prezalumab/AMG 557) in SLE, these findings further support the ICOS axis as a therapeutic target, with sICOS and haplotype status as candidate tools for pharmacodynamic monitoring and patient selection. Validation in larger, multi-assay, and multi-ethnic cohorts is required before any of these applications can be considered clinically actionable

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