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Correlation of TLR7 Gene Expression and Protein level with some immunological markers in Systemic Lupus Erythematosus Patients

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ABSTRACT

Systemic lupus erythematosus is an autoimmune chronic immune dysregulation disease accompanied by the production of autoantibodies. Recent research has revealed that TLR7 is among the main factors of SLE pathogenesis because it induces the activation of immune cells and triggers inflammatory cytokines. The aim of this proposed study is to assess the association between gene expression of TLR7 with serum levels of IL-39, IL-40, ANA and anti-dsDNA in patients with SLE in Iraqi females. This was conducted in a form of a case-control study whereby 70 SLE patients and 20 non-SLE cases were recruited. The levels of IL-39, IL-40, ANA, and anti-dsDNA were measured using ELISA whereas qRT-PCR was applied to measure the TLR7 gene expression. The findings demonstrated that there was a great rise in the expression of the TLR7 gene in SLE patients in comparison with controls (p value< 0.001). However, no significant difference was observed in the TLR7 protein content. Also, ANA and anti-dsDNA were significantly increased whereas IL-39, IL-40 were in a non-significantly increasing trend. There was a moderate positive correlation of TLR7 expression with these immune markers as revealed by Pearson correlation. The ROC curve analysis exhibited that there was a high diagnostic exactness concerning TLR7 gene expression. These results indicate that TLR7 could be one of the factors that cause the immune imbalance in SLE and could be a biomarker to monitor the disease.

KEYWORDS: Systemic Lupus Erythematosus, TLR7 gene expression, TLR7 protein level, Anti-dsDNA, Immune dysregulation.

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INTRODUCTION

Systemic lupus erythematosus is a complicated autoimmune disorder and systemic disease that has various clinical and hematological manifestations. It is a type of progressive development associated with the synthesis of autoantibodies, complement activation, and deposition of the immune complex in different tissues. This dysregulated immune response causes inflammation and damage to several Body parts, including the skin, kidneys, hematopoietic cells, brain, heart and lungs (1). The immunopathogenesis of SLE includes overproduction of autoantibodies including ANA and anti-dsDNA, immune complex formation and complement activation. Even though the exact etiology is not understood, genetic predisposition and environmental factors, which include ultraviolet radiation, hormones, drugs, and infections, are of critical importance to the loss of immune tolerance and subsequent autoimmunity development (2, 3).

In SLE pathogenesis, B cells are significant contributors, owing to the production of autoantibodies, antigen confirmation to T cells, and cytokine secretion. Partly, they are modified by various inborn immune receptors, especially Toll-like receptors (TLRs), which are pattern recognition receptors capable of detecting the conservation of the microbe and nucleic acid motifs and triggering the immune responses (2, 4). Among these, Toll-like receptor 7 (TLR7) is the essential pattern recognition receptor recognising single stranded RNA (ssRNA), activating downstream signalling mechanisms leading to secretion of type I interferon and leading to activation of self-reactive B cells (5, 6). Studies on both people and animals have shown that it has a role in the formation of SLE. Recent research has shown that mutations with gain-of-function in the TLR7 loci cause monogenic lupus cases. This means that the receptor's overexpression is a direct cause of the disease's severity (5). This can be explained by the truth that a study revealed that removal of TLR7 in B cells reduces lupus-like symptoms in murine models (7). Interestingly, TLR7 localized on the X chromosome and evades X-inactivation primarily in immune cells and may have contributed to the observed female predominance of SLE (8).

The sensitivities of TLR7 and TLR9 are not equal regarding the ability to detect infections caused by pathogens in the endosomes and nucleic acid sensing. Further developing on the previous theme, these receptors are found mostly in the plasmacytoid dendritic cells (pDCs) because they cause large production of interferon-alpha (IFN-α) and play a central part in the relation between the innate and adaptive immunity. Abnormal activation of TLR7 and TLR9 by hyperstimulation or dysregulation was proved to trigger autoimmune diseases as it spontaneously activates the immune cells such as SLE and rheumatoid arthritis (RA). As an illustration, excess activation of the TLR7 causes the body to produce more IFN and this exacerbates lupus nephritis in the mice models. When ligated, TLR7 and TLR9 activate the MyD88-dependent route, assembly of Myddosome with IRAK1, IRAK4 and TRAF6 (9). It leads to the expression of transcription factor IRF7 that favors type I IFN genes expression (2). Unmethylated DNA and RNA activate endosomal TLR, which causes the release of transcription factors (NF-kB), Leading to the release of cytokines that are pro-inflammatory and nucleic acid autoantibodies in SLE patients (10).

Besides classical cytokines, recently evidenced cytokines such as interleukin-39 (IL-39) and interleukin-40 (IL-40) were hypothesized to be involved in immune dysregulation associated with autoimmune disorders. IL-39, a component of the IL-12 family, is implicated in neutrophil survival and pro-inflammatory signaling (11). Whereas IL-40 that is the B cell-related cytokine coded by *C17orf99* locus is considered to participate in the control of B cell balance (12).

In line with this, This work aimed at analyzing the concentration of the protein and gene expression of TLR7 in female with SLE and determine its correlation with other immunological markers including ANA, anti-dsDNA, pro-inflammatory cytokines (IL-39, IL-40), blood cells counts (WBC, LYM, MON, BASO) and inflammatory phenomena (CRP, ESR) to explain the immunological-pathological processes involved in TLR7 and evaluate it as a diagnostic and prognostic marker in SLE.

MATERIALS AND METHODS

Design of the study and its participants

This case-control study included a total of 90 female participants, consisting of 70 patients diagnosed with SLE and 20 apparently healthy controls. Their ages ranged between 20 and 60 years old. Samples were collected during the period from September 2024 to January 2025 at Medical City in Baghdad, specifically from the Consultant of Arthritis, and the Lobby of Hematology and Arthritis at Baghdad Teaching Hospital. Diagnosis of SLE was confirmed by consultant physicians and the immunology unit based on the clinical criteria adopted by the hospital.

Sample Collection

Peripheral venous blood (10 mL) was collected under aseptic conditions using sterile syringes. Samples were distributed as listed below:

- 2 mL in EDTA tubes for complete blood count (CBC)
- 2 mL in 3.8% sodium citrate tubes for erythrocyte sedimentation rate (ESR)
- ~250 µL in microtubes containing 750 µL TRIzol for RNA extraction
- The remaining ~5 mL in gel separator tubes for serum isolation and ELISA assays

Hematological Analysis and c reactive protein (CRP) Levels

CBC parameters, including WBC, lymphocytes, monocytes, neutrophils, eosinophils, and basophils, were measured using the URIT-5500 hematology analyzer, which employs He-Ne laser-based flow cytometry. ESR was determined by the Westergren method, where citrated blood was placed vertically for 60 minutes and the sedimentation height recorded.

The serum CRP concentrations were measured through a latex enhanced turbidimetric immunoassay (CRP-Q, GIESSE Diagnostics Italy) performed by means of a fully automated biochemistry (GenoLabTEK, GenoTEK Inc., USA). Fixed time was used to read the absorbance at 540 nm and a calibration curve was used including 5 standard points.

Serological Assays (ELISA)

Serum concentrations of ANA, anti-dsDNA, IL-39, and IL-40 were quantified using ELISA kits, following the manufacturers' protocols:

- ANA: AESKULISA ANA-8S
- Anti-dsDNA: AESKULISA dsDNA-G
- IL-39 and IL-40: Sandwich ELISA kits

All assays involved standard procedures including reagent preparation, sample dilution, incubation, washing, substrate reaction, and absorbance reading at 450 nm. Values were interpolated from standard curves.

RNA Extraction and Gene Expression Analysis for TLR7

The extracted RNA was total RNA purified with the TransZol Up Plus RNA Kit (TransGen Biotech, China) using the manufacturer guidelines based on the peripheral blood samples of all participants. The NanoDrop 2000c spectrophotometer (Thermo Scientific, USA) was used to determine the purity and concentration of the RNA with an A260/A280 ratio that would fall within the acceptable range of 1.94-2.12 in the case of patients and 1.92-1.99 in the case of controls.

The reverse transcription was carried out by EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (TransGen Biotech, China). The reaction conditions were: 25°C 10min, 42°C 15min, and 85°C 5sec, reverse transcriptase inactivation protocol was observed. The resultant cDNA was directly used to study quantitative PCR.

The quantitative real-time PCR (qRT-PCR) was conducted on the Rotor-Gene Q machine (QIAGEN, Germany) with special primers of TLR7 and a housekeeping gene GAPDH. Thermal cycling conditions included 30 sec 94 °C (first denaturing) 40 cycles of 10 sec 94°C, 15 sec 60°C and 20 sec 72°C. A final analysis of melting curve was done between 55°C to 95°C to validate specificity in the amplification.

The expression of TLR7 was assessed by $2^{-\Delta\Delta Ct}$ where an endogenous control gene was GAPDH (13). The Ct values of the target and reference genes were obtained. Due to the melting temperature (Tm) of TLR7 amplicons of between 83 and 86 C, amplification specificity was indicated. In Table 1 the primers sequences of TLR7 and GAPDH are listed, validated on NCBI Primer-BLAST software.

Table 1: Primer Sequences Used for Quantification of TLR7 and GAPDH Gene Expression

Primer	Sequence (5′→3′ direction)	primer size bp	Ta °C
TLR7 (Gene Expression)			
Forward	GCTCTGTGGGAGTTCTGTCC	20	60
Reverse	ACCGTTTCCTTGAACACCTG	20	
***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase**			
Forward	GGCCTCCAAGGAGTAAGACC	20	60
Reverse	AGGGGTCTACATGGCAACTG	20	

Statistical Analysis

The analysis of data was done with IBM SPSS Statistics 29. Data were measured as means +/- SD. To examine the difference between groups, t-test was used, and the criterion of significance used was p-value < 0.05. Graph pad Prism version 9 was used to create graphical representations.

RESULTS

Comparison of Clinical and Hematological Markers Between SLE Patients and Controls

Comparison with control (as shown in Table 2) analysis of the level of blood CRP in SLE patients revealed a significant increase in average CRP levels in SLE patients in comparison to control (8.04 +/- 3.37 vs. 1.0 +/- 0.96, p value = 0.007). On the same note, ESR was relatively elevated in patients (31.9 +/- 13.24 vs. 10.6 +/- 3.30, p value = 0.001). The patients showed significantly low levels of WBC (7.0 ± 2.33 vs. 8.4 ± 2.29, p value = 0.01). Monocytes were significantly increased (7.3 ± 2.23 vs. 5.5 ± 0.66, p value = 0.001), while basophil counts were significantly decreased (0.4 ± 0.1 vs. 0.9 ± 0.27, p value = 0.001). Lymphocyte counts showed no significant difference between groups (33.1 ± 7.92 vs. 32.0 ± 9.33, p value = 0.5).

Table 2: Comparison of patients to controls in some immunological markers.

MARKER	SLE MEAN ± SD	CONTROL MEAN ± SD	P VALUE
CRP	8.04 ±3.37	1.0 ±0.96	0.007**
ESR	31.9 ±13.24	10.6 ±3.30	0.001**
WBC	7.0 ±2.33	8.4 ±2.29	0.01**
LYM	33.1 ±7.92	32.0 ±9.33	0.5 NS
MON	7.3 ±2.23	5.5 ±0.66	0.001**
BASO	0.4 ±0.1	0.9 ±0.27	0.001**

The values were expressed as mean ± SD; Statistical tests were made with T-test. SD: Std. Deviation, SE: Std. Error of Mean, NS, no significant difference.

3.2 Immunological Markers and TLR7 Protein Levels

Table 3 shows that IL-39 (3.5 ± 0.95 vs. 3.0 ± 1.29, p value = 0.2), IL-40 (26.6 ± 9.30 vs. 22.1 ± 8.95, p value = 0.3), and TLR7 protein levels (3.5 ± 1.07 vs. 3.9 ± 1.0, p value = 0.2) did not significantly differ among SLE patients and controls. However, anti-dsDNA levels (36.3 ± 10.81 vs. 21.1 ± 7.97, p value = 0.005) and ANA titers (38.6 ± 15.67 vs. 4.5 ± 1.29, p value = 0.001) were significantly higher in the patient group.

Table 3: Comparison between patients and control groups in IL-39, IL-40, TLR7 and Anti DNA.

MARKER	SLE MEAN ± SD	CONTROL MEAN ± SD	P-VALUE
IL-39	3.5 ±0.95	3.0 ±1.29	0.2 NS
IL-40	26.6 ±9.30	22.1 ±8.95	0.3 NS
TLR7	3.5 ±1.07	3.9 ±1.0	0.2 NS
ANTI DNA	36.3 ±10.81	21.1 ±7.97	0.005**
ANA	38.6 ±15.67	4.5 ±1.29	0.001**

The values were expressed as mean ± SD; Statistical tests were made with T-test. SD: Std. Deviation, SE: Std. Error of Mean, NS, no significant difference.

Real-time PCR measuring expression of TLR7.

Analysis of the expression of TLR7 was analyzed using qRT-PCR in 70 SLE patients and 20 control subjects. The mean Ct values for TLR7 were 19.29 ± 2.90 in patients and 22.80 ± 2.40 in controls. Applying the 2^-ΔΔCt method, a 3.50-fold upregulation of TLR7 expression was found in patients than in controls (p value = 0.001) (Table 4). Each run's plots, including amplification plots and dissociation curves, were recorded.

Table 4: Comparison of TLR7 gene in Ct, ΔCt, ΔΔct and fold values between investigation groups.

GROUPS	MEANS CT OF *TLR7*	MEANS CT OF *GAPDH*	ΔCT (MEANS CT OF *TLR7*)	ΔΔCT	FOLD (2^-ΔΔCT)	P VALUE
PATIENTS	19.286	21.6395	-2.3535	-1.4825	3.50	0.001**
CONTROL	22.796	23.667	-0.871	0.0000	1.11	

3.4 ROC Curve Analysis of TLR7 Expression

A receiver operating characteristic curve was built so as to evaluate the diagnostic use of TLR7 gene expression in distinguishing SLE patients from healthy controls. The test found an area under the curve (AUC) of 0.88 (95% CI: 0.80-0.95), indicating good discriminative ability (Figure 1). At an optimal cutoff value of 1.835 (2^$^{-\Delta\Delta ct}$), TLR7 expression achieved a sensitivity of 80.0% and specificity of 95.0% (Table 5)

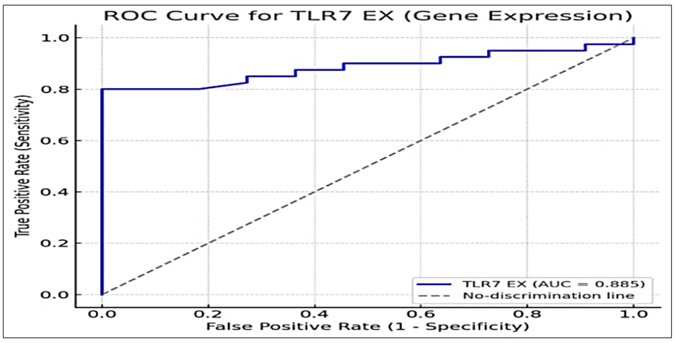

Figure 1: ROC Curve Displaying Diagnostic Value of TLR7 Gene Expression to Differentiate between SLE Patients and Healthy Controls.

Table 5: ROC Curve Analysis of TLR7 Gene Expression for Discriminating SLE Patients from Controls.

Area	Std. Error^{a}	Asymptotic Lower Sig.^{b}	The best Cut off	Sensitivity %	Specificity%
0.885	.046	.000	1.835	80	95

Correlation Analysis Between Immunological and TLR7 in SLE Patients

Pearson correlation coefficient values (Table 7) showed that there is a moderate positive correlation between IL-40 and TLR7 protein (r = 0.554, p value < 0.001) and IL-39 and TLR7 protein (r = 0.414, p value < 0.001). There was also a moderate positive relationship observed between TLR7 gene expression and ANA level (r = 0.416, p value = 0.002).

The TLR7 gene expression was not strongly associated with anti-dsDNA (r = 0.011, p value = 0.939) and neither did the TLR7 protein levels with TLR7 gene expression (r = 0.083, p value = 0.561), which indicates a potential post-transcription regulation. Other correlations were merely weak or very weak and statistically non-significant (IL-39 or IL-40 with the anti-dsDNA and ANA).

Table 6: Pearson Correlation Matrix Between Immunological and TLR7 in SLE Patients.

CORRELATED VARIABLES	R-VALUE	P VALUE	STRENGTH OF CORRELATION
IL-39 & IL-40	0.432	<0.001	Moderate
IL-39 & TLR7 (PROTEIN)	0.414	<0.001	Moderate
IL-39 & ANTI-DSDNA	-0.014	0.897	Very weak
IL-39 & TLR7 EX. (GENE)	0.104	0.470	Very weak
IL-39 & ANA	0.171	0.108	Weak
IL-40 & TLR7 (PROTEIN)	0.554	<0.001	Moderate to Strong
IL-40 & ANTI-DSDNA	0.002	0.987	Very weak
IL-40 & TLR7 EX (GENE)	0.114	0.424	Very weak
IL-40 & ANA	0.118	0.270	Very weak
TLR7 (PROTEIN) & ANTI-DSDNA	-0.201	0.057	Weak
TLR7 (PROTEIN) & TLR7 EX. (GENE)	0.083	0.561	Very weak
TLR7 (PROTEIN) & ANA	-0.044	0.679	Very weak
ANTI-DSDNA & TLR7 EX. (GENE)	0.011	0.939	Very weak
ANTI-DSDNA & ANA	0.237	0.024	Weak
TLR7 EX (GENE) & ANA	0.416	0.002	Moderate

Pearson correlation coefficient (r) was adopted to determine the magnitudes and direction of the existence of linear relations between desired immunological and biochemical indicators. The interpretation of correlation strength followed these thresholds:

- Very weak ($r < 0.3$)
- Weak ($0.3 \leq r < 0.5$)
- Moderate ($0.5 \leq r < 0.7$)
- Strong ($0.7 \leq r < 0.9$)
- Very strong ($r \geq 0.9$)

DISCUSSION

SLE is a disease that is characterized with chronic inflammation and this was reflected by the acute increase of CRP and ESR in this study. These biomarkers are valid and easy accessibility measures of the disease activity (14). Depending on the ACR criteria, leukopenia SLE can be caused by bone marrow suppression or the immune-mediated destruction (15). There had also been a dramatic reduction in the level of basophils during the active disease stage that could be attributed to the migration of this type of cell to the inflammatory areas. This confirms the view of (16). Since Jiang et al., (2021) and Dossybayeva et al., (2020) are negatively correlated with disease activity after therapy course (17, 18). Whereas the active SLE tends to be associated with lymphopenia (19), it was not relevant to our study, potentially due to the effects of treatments. Monocytosis was associated with the activity and with an elevated interferon-stimulated gene (20). Anti-dsDNA and ANA were also increased significantly, which are known to be diagnostic of usefulness. ANA has high sensitivity, whereas anti-dsDNA has specificity, and particularly in renal conflict. These findings are in agreement with those of (21).

In this research, the amount of IL-39 in the serum of SLE patients has been found to be a little bit higher than in the control group but this did not achieve even the level of significance. Nevertheless, some previous reports viewed that the IL-39 contributes to the immunopathogenesis of SLE which means that it could be produced by B cells, and contributes to their activation and development of neutrophils, as well as production of BAFF. Despite scarce and partly contradictory human data, IL-39 can be useful as a highly specific, but insensitive biomarker in combination with other immune biomarkers (22, 23).

This study also found the serum IL-40 was higher levels in SLE patients vs. control, but not significant. Il-40 is an innovative cytokine that regulates B cells and production of antibodies, and which has been implicated in chronic inflammation and disease activity in SLE (24). Although it does not show much discriminatory power in this study, its role is yet to be better characterized, especially in studies following patients over time, where changes may help more in the diagnosis.

Interestingly, while TLR7 gene expression has been reported to be Heightened in SLE patients, our ELISA measurements indicated slightly reduced protein levels in patients compared to controls. This discrepancy may reflect post-transcriptional regulation or increased protein degradation under inflammatory conditions. Such gene–protein expression disparities have been widely documented, where mRNA levels do not necessarily correlate with protein plenty (25). A gene expression analysis revealed a huge upregulation of TLR7 (3.5-fold, $p = 0.001$) in line with previously documented functions of this receptor as an activator of type I IFNs signaling and autoreactive B cells (26). Female predominance could be due to its X-chromosomal location and avoiding the inactivation mechanism (8, 27). The overexpression of TLR7 enhances the production of ANA in line with (28, 29).

The ROC analysis indicated that TLR7 has a high level of diagnostic activity (AUC 0.885, 80% sensitivity, 95% specificity), indicating its usefulness as a molecular marker that can be used especially in the group of autoimmune diseases found mostly in women (8, 30, 31). TLR7 expression was associated with ANA abundance and supported its promotion of autoreactive B cells (29, 32). Scientifically, this reinforced the effect driven by TLR7 in the autoimmune cascade. There was also an important positive correlation in the expression of TLR7 with the levels of IL-39 and IL-40, which shows a potential involvement of TLR7-mediated B cell activation in the production of those two cytokines. Taking into account the fact that the IL-40 is B cell related cytokine linked with the presence of autoimmunity and decreased upon B cell depletion (Navrátilová et al., 2021), this confirms the function of TLR7 in autoimmune reactions through cytokine-mediated mechanisms. Moderate correlation among IL-39 and IL-40 ($r = 0.432$) could indicate this co-expression in inflammatory environments, as also reported in cases of ankylosing spondylitis (33), and perhaps is a factor in SLE. The positive relation of TLR 7 and ANA ($r = 0.416$) confirms the role of the former in stimulating antinuclear antibodies (34). Lastly, that ANA and anti-dsDNA levels are correlated so well demonstrates how mutually beneficial they are in terms of diagnosing and monitoring a disease because they both reflect antigen-stimulated autoimmune responses, with B and T cell dysregulation (35).

LIMITATIONS AND FUTURE PATHWAYS

A number of Constraints were related with this study. First, the sample size is not particularly large and the findings are recruited in a single geographic location and this may limit the extent of the generalizability of the results. Second, the study structure (cross-sectional one) does not provide any possibility to infer about the cause or time aspect. Third, genetic polymorphisms of TLR7 or downstream signaling pathways that may further elucidate the mechanistic role of TLR7 in SLE were not examined by the study. Besides, the study was limited to female patients only, which restricts the possibility to investigate sex-based variations in immunology.

It is possible to recommend that future studies should take into account metacentric studies involving more diverse and larger populations of patients of both sexes to increase the external validity of the results. Longitudinal research studies are also needed to measure the predictive nature of TLR7 expression and proteins level on disease progression and therapeutic outcome. Besides, the study of TLR7 gene polymorphisms and their influence on cytokine networks and B cell activation may give more insight into the mechanism. Finally, The interventional research of TLR7 pathway can give some new perspectives in developing intervention strategies in treating SLE.

CONCLUSION

The study results indicate that there is a Considerable rise of the TLR7 gene in SLE in a particular group of Iraqi women but the TLR7 protein level remains unchanged in serum. ANA and anti-dsDNA concentrations were greatly elevated in the patients, and IL-39 and IL-40 showed an insignificant increase in the level. A reasonable expression of TLR7 was found in correlating with IL-39, IL-40, and ANA concentrations, validating the possibility of having TLR7 making a contribution to immune activation in relation to SLE. Nevertheless, TLR7 showed no correlation of importance with anti-dsDNA. The ROC analysis shows that the diagnostic value of measuring the expression of the TLR 7 gene may indicate its potential application as a diagnostic factor in detection and follow-up of SLE. These findings provide insight into the immunopathogenesis of SLE and warrant further research into TLR7-targeted therapies.

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Conflict of interest: The authors declare no conflict of interest in this study.

Ethical Considerations: The Scientific Research Ethics Committee of the College of Education for Pure Science/ the University of Mosul and the Iraqi Ministry of Health approved the study. All participants gave verbal consent and verbal information, in following the instructions of the Announcement of Helsinki prior to sample collection.

REFERENCES

1. Mohamed A, Nadra E, Najla E, Wasim E, Retaj A, Sondos G. Hematological Disorders of Systemic Lupus Erythematosus: A Cross-Sectional Study. Khalij-Libya Journal of Dental and Medical Research. 2025:97-102. doi: 10.47705/kjdmr.25911015.
2. Baek W-Y, Choi Y-S, Lee S-W, Son I-O, Jeon K-W, Choi S-D, et al. Toll-like Receptor Signaling Inhibitory Peptide Improves Inflammation in Animal Model and Human Systemic Lupus Erythematosus. International Journal of Molecular Sciences. 2021;22(23):12764. PubMed PMID: doi:10.3390/ijms222312764.
3. Kahya HFH, Mahmood MT. The Relationship between Stress and Immune Function: A Review Article. Commagene Journal of Biology. 2025;9(1):112-22. doi: 10.31594/commagene.1641548.
4. Wen L, Zhang B, Wu X, Liu R, Fan H, Han L, et al. Toll-like receptors 7 and 9 regulate the proliferation and differentiation of B cells in systemic lupus erythematosus. Frontiers in Immunology. 2023;Volume 14 - 2023. doi: 10.3389/fimmu.2023.1093208.
5. Scofield RH, Wren JD, Lewis VM. The toll like receptor 7 pathway and the sex bias of systemic lupus erythematosus. Frontiers in Immunology. 2025;Volume 16 - 2025. doi: 10.3389/fimmu.2025.1479814.
6. Brown GJ, Cañete PF, Wang H, Medhavy A, Bones J, Roco JA, et al. TLR7 gain-of-function genetic variation causes human lupus. Nature. 2022;605(7909):349-56. doi: 10.1038/s41586-022-04642-z.
7. 7. Kalliolias GD, Basdra EK, Papavassiliou AG. Targeting TLR Signaling Cascades in Systemic Lupus Erythematosus and Rheumatoid Arthritis: An Update. Biomedicines. 2024;12(1). Epub 20240109. doi: 10.3390/biomedicines12010138. PubMed PMID: 38255243; PubMed Central PMCID: PMCPMC10813148.
8. Souyris M, Cenac C, Azar P, Daviaud D, Canivet A, Grunenwald S, et al. TLR7 escapes X chromosome inactivation in immune cells. Science immunology. 2018;3(19). doi: 10.1126/sciimmunol.aap8855. PubMed PMID: 29374079.
9. De Spiegeleer A, Kahya H, Crombez L, Descamps A, Rössler R, Kressig RW, et al. Potential role of statins in treatment of acute sarcopenia. Medical Hypotheses. 2023;177:111111. doi: https://doi.org/10.1016/j.mehy.2023.111111.
10. Jassim AS, Auda IG, Ali EN. Toll-like receptor-7 gene polymorphism at 3− UTR (rs3853839) in relation to Systemic Lupus Erythematosus pathogenesis in Iraqi patients. Gene Reports. 2023;33:101822. doi: https://doi.org/10.1016/j.genrep.2023.101822.
11. Wang X, Liu X, Zhang Y, Wang Z, Zhu G, Han G, et al. Interleukin (IL)-39 [IL-23p19/Epstein–Barr virus-induced 3 (Ebi3)] induces differentiation/expansion of neutrophils in lupus-prone mice. Clinical and Experimental Immunology. 2016;186(2):144-56. doi: 10.1111/cei.12840.
12. Navrátilová A, Andrés Cerezo L, Hulejová H, Bečvář V, Tomčík M, Komarc M, et al. IL-40: A New B Cell-Associated Cytokine Up-Regulated in Rheumatoid Arthritis Decreases Following the Rituximab Therapy and Correlates With Disease Activity, Autoantibodies, and NETosis. Frontiers in Immunology. 2021;Volume 12 - 2021. doi: 10.3389/fimmu.2021.745523.
13. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods. 2001;25(4):402-8. doi: https://doi.org/10.1006/meth.2001.1262.
14. Akdogan MR, Melikoglu MA. A potential biomarker of disease activity in systemic lupus erythematosus, systemic immune-inflammation index. North Clin Istanb. 2024;11(2):115-9. Epub 20240422. doi: 10.14744/nci.2023.90132. PubMed PMID: 38757099; PubMed Central PMCID: PMCPMC11095327.

15. Fayyaz A, Igoe A, Kurien BT, Danda D, James JA, Stafford HA, et al. Haematological manifestations of lupus. Lupus Sci Med. 2015;2(1):e000078. Epub 2015/04/11. doi: 10.1136/lupus-2014-000078. PubMed PMID: 25861458; PubMed Central PMCID: PMCPMC4378375.
16. Liang P, Tang Y, Fu S, Lv J, Liu B, Feng M, et al. Basophil count, a marker for disease activity in systemic lupus erythematosus. Clin Rheumatol. 2015;34(5):891-6. Epub 20141119. doi: 10.1007/s10067-014-2822-9. PubMed PMID: 25403252.
17. Jiang Y, Chen J, Zhao Y, Liu Y, Xu H, Mo X. Basophils as a potential marker of lupus nephritis by flow cytometry. Future science OA. 2021;7(5):Fso690. Epub 2021/05/29. doi: 10.2144/fsoa-2020-0212. PubMed PMID: 34046192; PubMed Central PMCID: PMCPMC8147755.
18. Dossybayeva K, Abdukhakimova D, Poddighe D. Basophils and Systemic Lupus Erythematosus in Murine Models and Human Patients. Biology. 2020;9(10). Epub 2020/09/27. doi: 10.3390/biology9100308. PubMed PMID: 32977704; PubMed Central PMCID: PMCPMC7598686.
19. Faddah S, Elwakd M, Aboelenein A, Hussein M. Lymphopenia and systemic lupus erythematosus, a preliminary study: Correlation with clinical manifestations, disease activity and damage indices. The Egyptian Rheumatologist. 2014;36(3):125-30. doi: https://doi.org/10.1016/j.ejr.2014.01.005.
20. Zhang J, Zhuang W, Li Y, Deng C, Xuan J, Sun Y, et al. Bioinformatic analysis and experimental verification reveal expansion of monocyte subsets with an interferon signature in systemic lupus erythematosus patients. Arthritis Research & Therapy. 2025;27(1):96. doi: 10.1186/s13075-025-03560-5.
21. Wichainun R, Kasitanon N, Wangkaew S, Hongsongkiat S, Sukitawut W, Louthrenoo W. Sensitivity and specificity of ANA and anti-dsDNA in the diagnosis of systemic lupus erythematosus: A comparison using control sera obtained from healthy individuals and patients with multiple medical problems. Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand. 2013;31:292-8. doi: 10.12932/AP0272.31.4.2013.
22. Wang X, Wei Y, Xiao H, Liu X, Zhang Y, Han G, et al. A novel IL-23p19/Ebi3 (IL-39) cytokine mediates inflammation in Lupus-like mice. European journal of immunology. 2016;46(6):1343-50. Epub 2016/03/29. doi: 10.1002/eji.201546095. PubMed PMID: 27019190; PubMed Central PMCID: PMCPMC11334612.
23. Ahmadi P, Janzadeh A, Honardoost M, Taherkhani S, Doyle D, Mojarad N. The role of IL-39 in autoimmune diseases: From general to immunopathogenesis. Cytokine. 2025;192:156969. Epub 20250528. doi: 10.1016/j.cyto.2025.156969. PubMed PMID: 40440970.
24. Dabbagh-Gorjani F. A Comprehensive Review on the Role of Interleukin-40 as a Biomarker for Diagnosing Inflammatory Diseases. Autoimmune Dis. 2024;2024:3968767. Epub 20240301. doi: 10.1155/2024/3968767. PubMed PMID: 38464677; PubMed Central PMCID: PMCPMC10923619.
25. Mohammed, A. A., Barale, S. S., Dhotare, P. S., & Sonawane, K. D. (2025). Polymyxin-B as a Novel Inhibitor of Amyloid Beta Aggregation: Computational Insights and Experimental Validation. *Journal of Molecular Structure*, 143738.
26. Celhar T, Lu HK, Benso L, Rakhilina L, Lee HY, Tripathi S, et al. TLR7 Protein Expression in Mild and Severe Lupus-Prone Models Is Regulated in a Leukocyte, Genetic, and IRAK4 Dependent Manner. Frontiers in Immunology. 2019;Volume 10 - 2019. doi: 10.3389/fimmu.2019.01546.
27. Scofield RH, Wren JD, Lewis VM. The toll like receptor 7 pathway and the sex bias of systemic lupus erythematosus. Front Immunol. 2025;16:1479814. Epub 2025/03/07. doi: 10.3389/fimmu.2025.1479814. PubMed PMID: 40051623; PubMed Central PMCID: PMCPMC11882868.
28. Rubtsov AV, Rubtsova K, Kappler JW, Marrack P. TLR7 drives accumulation of ABCs and autoantibody production in autoimmune-prone mice. Immunol Res. 2013;55(1-3):210-6. doi: 10.1007/s12026-012-8365-8. PubMed PMID: 22945807; PubMed Central PMCID: PMCPMC3935605.
29. Kamble, S. A., Barale, S. S., Mohammed, A. A., Paymal, S. B., Naik, N. M., & Sonawane, K. D. (2024). Structural insights into the potential binding sites of Cathepsin D using molecular modelling techniques. Amino acids, 56(1), 33. https://doi.org/10.1007/s00726-023-03367-1
30. Huret C, Ferrayé L, David A, Mohamed M, Valentin N, Charlotte F, et al. Altered X-chromosome inactivation predisposes to autoimmunity. Sci Adv. 2024;10(18):eadn6537. Epub 20240503. doi: 10.1126/sciadv.adn6537. PubMed PMID: 38701219; PubMed Central PMCID: PMCPMC11068014.
31. Dudkova M, Petrackova A, Radvansky M, Skacelova M, Videman J, Manakova J, et al. Blood gene expression of Toll-like receptors in SLE patients with lupus nephritis or neuropsychiatric systemic lupus erythematosus. Arthritis Res Ther. 2025;27(1):41. Epub 20250226. doi: 10.1186/s13075-025-03512-z. PubMed PMID: 40011954; PubMed Central PMCID: PMCPMC11863772.
32. Chodisetti SB, Fike AJ, Domeier PP, Singh H, Choi NM, Corradetti C, et al. Type II but Not Type I IFN Signaling Is Indispensable for TLR7-Promoted Development of Autoreactive B Cells and Systemic Autoimmunity. J Immunol. 2020;204(4):796-809. Epub 20200103. doi: 10.4049/jimmunol.1901175. PubMed PMID: 31900342; PubMed Central PMCID: PMCPMC7002260.
33. Jaber AS, Ad'hiah AH. A novel signature of interleukins 36α, 37, 38, 39 and 40 in ankylosing spondylitis. Cytokine. 2023;162:156117. doi: https://doi.org/10.1016/j.cyto.2022.156117.
34. Celhar T, Fairhurst AM. Toll-like receptors in systemic lupus erythematosus: potential for personalized treatment. Front Pharmacol. 2014;5:265. Epub 20141208. doi: 10.3389/fphar.2014.00265. PubMed PMID: 25538618; PubMed Central PMCID: PMCPMC4258990.
35. Pisetsky DS. Evolving story of autoantibodies in systemic lupus erythematosus. Journal of Autoimmunity. 2020;110:102356. doi: https://doi.org/10.1016/j.jaut.2019.102356.