TOLL-LIKE RECEPTORS SIGNATURE IN ACTIVE LUPUS NEPHRITIS

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BACKGROUND

A number of studies have demonstrated that different members of the TLR family are involved in the pathogenesis of SLE. The expression of TLRs varies in different clinical manifestations of the disease. Some of the TLRs (TLR7, TLR8) are potential therapeutic targets for monoclonal antibodies. The role of innate immunity in the SLE development is already known. However, there is limited knowledge regarding the differences and similarities in TLR members based on specific organ damage. This study focused on the possible differences between the expression of TLR members in SLE with severe disease manifestations such as active lupus nephritis (LN) with no LN patients. The study analysed the gene expression of TLR family members in a clinically defined group of SLE patients in order to evaluate the potential of TLRs for the diagnosis of organ involvement. Objective of the study was to determine differences in the blood innate gene expression signature in systemic lupus erythematosus (SLE) across organ manifestations and disease activity, with a focus on lupus nephritis (LN).

METHODS

The Toll-like receptor family (TLR 1-10) mRNA expression was investigated in peripheral blood mononuclear cells from SLE patients (n=74) and healthy controls (n=34). We compared the group of patients with histologically confirmed active LN with no LN patients. The expression of TLRs mRNA was performed by RT-qPCR using high-throughput SmartChip Real-Time- qPCR system (WaferGen). Multivariate analysis was used for data analysis and nonparametric statistics to assess the association between TLRs and disease activity and severity. All SLE patients enrolled in the study met the 2019 EULAR/ACR classification criteria for SLE . Disease activity was quantified using the Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI)]. The organ damage accumulated since disease onset was assessed using the Systemic Lupus International Collaborating Clinics American College of Rheumatology Damage Index (SLICC/ACR DI).

RESULTS

Table 1. Demographic and Clinical Characteristics of Enrolled Patients

	SLE patients	Control group
Number of patients	74	34
Age mean (range)	40 (19-74)	40 (24-50)
Female/Male	65/9	29/5
		SLE patients (n=74)
Duration of disease (years) mean (min-max)		11.3 (1–38)
SELENA-SLEDAI mean (min-max)		7.0 (0–43)
SLICC/ACR DI mean (min-max)		1.21 (0–8)
C3 level (g/l) mean (min-max)		0.88 (0.2–1.72)
C4 level (g/l) mean (min-max)		0.14 (0.06–0.3)
Anti ds DNA IgG (IU/ml) mean (min-max)		68.38 (<12.5– >200)
Anti-nucleosome antibodies (IU/ml) (min-max)		111.6 (1.7– >200)
Active LN-SLE / Inactive LN-SLE / No LN-SLE group		13/14/47
Active LN – Proteinuria (mean - g/day)		1.5 (0.36–6.03)
- Proteinuria (>0.5g/day) Hematuria/Pyuria		11/4/7
NPSLE - according ACR (yes/no)		9/65
Seizures / Cerebrovascular accident / Organic brain syndrome / Headache		3/2/3/1

Table 2. Relative TLRs mRNA expression according to SLE subgroups and healthy controls

A) SLE vs. healthy controls

Gene	Mean (95 % CI)		P-value
	Controls	SLE	
TLR1	0.0482 (0.005–0.107)	0.053 (0.006–0.176)	0.599
TLR2	0.039 (4.75 × 10^{-5}–0.208)	0.040 (1.7 × 10^{-4}–0.213)	0.590
TLR3	0.002 (2.51× 10^{-5}–0.017)	0.0009 (2.46× 10^{-5}–0.013)	0.122
TLR4	0.044 (0.008–0.118)	0.081 (0.011–0.503)	0.012*
TLR5	0.021 (3.94× 10^{-5}–0.76)	0.020 (2.81× 10^{-5}–0.103)	0.976
TLR6	0.03 (6.4 × 10^{-4}–0.117)	0.041 (0.004–0.230)	0.323
TLR7	0.014 (1.56 × 10^{-4} –0.039)	0.019 (6.63× 10^{-5}–0.104)	0.655
TLR8	0.043 (0.004–0.190)	0.047 (2.42 × 10^{-4}–0.241)	0.682
TLR9	0.010 (2.5× 10^{-5}–0.022)	0.012 (4.69× 10^{-5}–0.073)	0.408
TLR10	0.009 (5.0× 10^{-4}–0.022)	0.006 (2.71× 10^{-5}–0.430)	0.0007*

B) Active LN-SLE vs. No LN-SLE

Gene	Mean (95 % CI)		P-value
	Active LN-SLE	No LN-SLE	
TLR1	0.069 (0.017–0.162)	0.051 (0.006–0.175)	0.03*
TLR2	0.072 (0.007–0.213)	0.030 (1.6×10^{-4}–0.117)	0.007*
TLR3	5.1×10^{-4} (5.0×10^{-5}–0.003)	0.001 (2.5×10^{-5}–0.014)	0.4
TLR4	0.110 (0.033–0.503)	0.079 (0.015–0.325)	0.11
TLR5	0.031 (5.0×10^{-5}–0.003)	0.017 (4.7×10^{-5}–0.102)	0.12
TLR6	0.044 (0.015–0.097)	0.036 (0.004–0.168)	0.05*
TLR7	0.019 (0.006–0.0385)	0.0188 (6.6×10^{-5}–0.241)	0.15
TLR8	0.062 (0.008–0.167)	0.041 (2.4×10^{-4}–0.039)	0.03*
TLR9	0.014 (5.0×10^{-5}–0.061)	0.009 (4.7×10^{-5}–0.39)	0.55
TLR10	0.006 (5.0×10^{-5}–0.017)	0.006 (2.7×10^{-5}–0.061)	0.78

P-values less than 0.05 were considered statistically significant and are indicated by an asterisk. SLE Systemic lupus erythematosus, LN lupus nephritis, TLR Toll like receptor

Table 3. Correlations of Toll like receptors (TLR) mRNA expression levels

	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10
TLR1	1.000	0.397*	0.106	0.679*	0.276*	0.201	0.539*	0.703*	-0.044	0.270*
TLR2	0.397*	1.000	0.120	0.150	0.646*	-0.132	0.156	0.472*	0.053	0.151
TLR3	0.106	0.120	1.000	0.066	0.150	-0.067	-0.017	-0.067	0.264*	-0.047
TLR4	0.679*	0.150	0.066	1.000	-0.007	0.383*	0.554*	0.635*	-0.250*	-0.046
TLR5	0.276*	0.646*	0.150	-0.007	1.000	-0.048	-0.024	0.393*	0.165	0.331*
TLR6	0.201	-0.132	-0.067	0.383*	-0.048	1.000	0.171	0.463*	0.052	-0.013
TLR7	0.539*	0.156	-0.017	0.554*	-0.024	0.171	1.000	0.570*	-0.094	0.225
TLR8	0.703*	0.472*	-0.067	0.635*	0.393*	0.463*	0.570*	1.000	-0.066	0.207
TLR9	-0.044	0.053	0.264*	-0.250*	0.165	0.052	-0.094	-0.066	1.000	0.381*
TLR10	0.270*	0.151	-0.047	-0.046	0.331*	-0.013	0.225	0.207	0.381*	1.000

TLR Toll like receptor, Spearman correlation coefficients marked with asterisk are significant at the 0.05 level.

Fig.1 Toll like receptors mRNA relative expression – SLE vs. healthy controls

Abbreviations: TLR Toll-like receptor, SLE Systemic lupus erythematosus

Note: The relative mRNA expression of the TLR family members was compared between patients with SLE and healthy controls. Compared to healthy controls, the relative mRNA expression of TLR4 was upregulated in the SLE group (0.044 vs 0.081, p=0.012, Table 2A). Conversely, TLR10 expression was significantly downregulated in the SLE group compared to healthy controls (0.009 vs. 0.006, p=0.0007, Table 2A). Furthermore, increased expression of TLR6 and TLR7 and decreased expression of TLR3 were observed, although without reaching statistical significance.

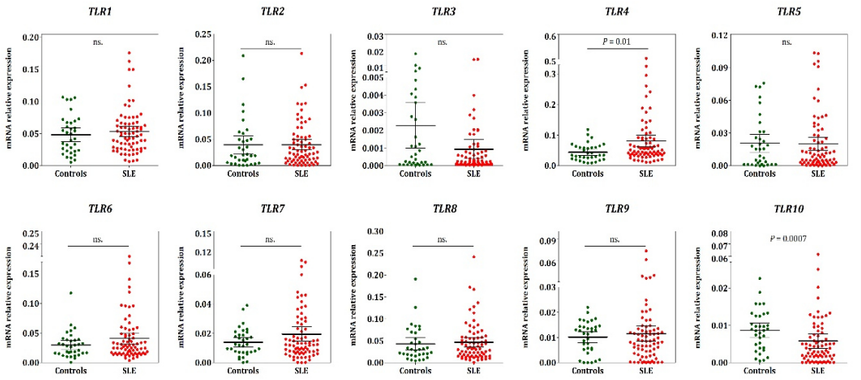


Fig.2 Toll like receptors mRNA relative expression – no LN SLE vs. active LN SLE patients

Abbreviations: TLR Toll-like receptor, LN Lupus nephritis

Note: To investigate the relationship between innate immune gene expression and clinical manifestations, we conducted a comparison between SLE patients without renal impairment and patients with active LN according to the renal SLEDAI. For TLR1 (0.051 vs. 0.069, p=0.03), TLR2 (0.03 vs. 0.075, p=0.007), TLR6 (0.036 vs. 0.044, p=0.05) and TLR8 (0.041 vs. 0.062, p=0.03) we found a significant mRNA upregulation (Table 2B). TLR3 expression was found to be reduced in the active LN group, although this did not reach statistical significance.

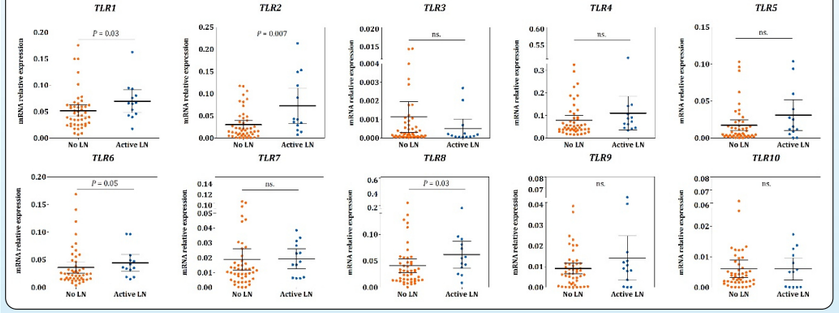


Fig3:

ROC analysis of TLR1 and TLR2 mRNA expression

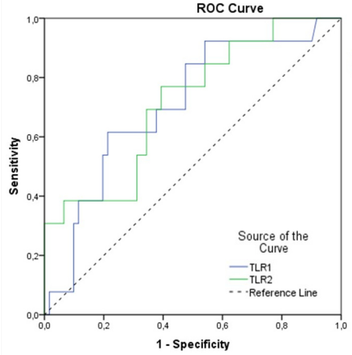
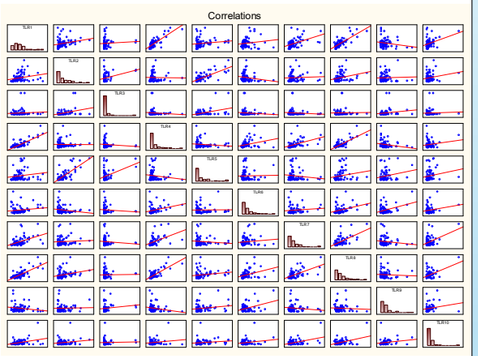


Fig.4

Correlations of TLR receptor mRNA expression levels



Abbreviations: TLR Toll-like receptor, ROC curve Receiver operating characteristic curve

Note: ROC analysis indicated that TLR1 and TLR2 were potential predictive markers for active LN among the studied TLRs. TLR1 demonstrated an area under the curve (AUC) of 0.707 with a 95% confidence interval (CI) of 0.556-0.857 and a p-value of 0.020. TLR2 exhibited an AUC of 0.715 with a 95% CI of 0.564-0.866 and a p-value of 0.015. AUC levels above 0.7 were regarded as a reasonable discriminating value for predicting diagnostic tests.

CONCLUSION

Our study revealed differences in TLR expression, particularly upregulated TLR1, 2, 6 and TLR8, in patients with active LN comparing to no LN patients. The multiple mutual relations of TLRs demonstrate the activation of innate immunity in SLE and suggest promising targets for future therapies or diagnostics.

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