RESEARCH ARTICLE

Anti-Ribosomal P Protein Antibody, Anti-Nuclear Antibody, and Tumor Necrosis Factor-α Polymorphism Association with Chronic Kidney Disease Risk in SLE Patients

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ARTICLE INFO	ABSTRACT
  *Corresponding Author: pstm.hadi.a.hadi@jmu.edu.iq	Systemic lupus erythematosus (SLE) is a multifaceted autoimmune disease affecting various organs characterized by autoantibodies and multi-organ involvement. SLE frequently includes kidney complications that can significantly worsen outcomes, leading to chronic kidney disease (CKD). This study aims to investigate whether anti-ribosomal P and ANA antibodies alongside a TNF-α gene variation (rs1800629), influence the development of CKD in patients with SLE. A case-control study of 89 SLE patients. They measured ANA and Anti-Rib P antibodies by ELISA and TNF-a (rs1800629) polymorphism by Allele-specific PCR. Eighty-nine (89) patients were enrolled in this study, The LN was developed in 36 (40%) patients. The average of Anti-Rib-P positivity was 14.28 % in non-CKD LN more than CKD-LN (13.63%) and SLE (3.77%) which is highly significant in the two LN groups than the SLE group and in the non-CKD LN group than the CKD-LN group (LSD = 0.857, alph = 0.05). At the same time, the ANA positivity was (86.36%) in CKD-LN more than in non-CKD LN (78.57%) and SLE (41.51%) with highly significant in CKD-LN than non-CKD LN and SLE patients and highly significant in non-CKD LN than SLE patients (LSD = 4.195, alpha = 0.05). Regarding the TNF-a (rs1800629) gene, the AA genotype was the low frequency (13.33%) present only in the CKD-LN group. GA genotype was the most commonly present in CKD-LN (86.66%), non-CKD LN (100%), SLE (83.33%), and the health group (100%). In contrast, the GG genotype presents in the SLE group (16.66%), a highly significant difference was among groups (LSD = 4.92). The anti-Rib-P antibody is associated with LN and has no important correlation with chronic kidney disease. In contrast, ANA is associated with chronic kidney disease and could be a prognostic and predictor marker in LN patients. At the same time, the AA genotype and A allele are implicated with CKD and could worse renal outcomes in LN patients..

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune illness that exhibits clinical diversity and affects several systems. It is characterized by autoantibodies, involvement of multiple organs, and a chronic course of the disease. Renal involvement is a prevalent sign of SLE, with Lupus Nephritis (LN) being seen in about 60% of patients throughout the progression of SLE. Renal involvement is usually the major cause of mortality since this may create significant problems and

can lead to Chronic Kidney Disease (CKD) as well as end-stage renal illness. The clinical manifestations of this condition range from mild, asymptomatic, gradually increasing proteinuria to quickly advancing glomerulonephritis that necessitates either temporary or permanent dialysis (1) (2).

Antinuclear antibodies (ANA) play a crucial role in diagnosing systemic lupus erythematosus (SLE) and are regarded as key diagnostic and entry criteria for the disease (3). The presence of ANA, especially IgG-ANA, is a diagnostic characteristic of systemic lupus erythematosus (SLE) and other systemic autoimmune illnesses, playing a significant part in developing this disease (4). Anti-ribosomal P (Anti-Rib-P) autoantibodies have a strong affinity for SLE patients and measuring these antibodies might enhance the accuracy of diagnosing SLE. There was an observed correlation between Caucasian ethnicity and decreased levels of anti-Rib-P antibodies (5). The occurrence of anti-Rib-P antibodies in SLE patients ranges from 15% to 40% and are influenced by factors such as ethnicity, illness activity, and detecting technique. These antibodies are strongly linked to Lupus nephritis, liver involvement, neuropsychiatric symptoms, lymphopenia, arthritis, and juvenile SLE (6). An in vivo study showed that in lupus-prone mice, anti-P is a nephrogenic antibody that promotes the onset of glomerulonephritis (7).

LN patients having anti-P showed a greater incidence of active nephritis than those without anti-P, and anti-P was missing during times of illness re-mission (8). According to a study, anti-P was linked to improved long-term renal outcomes and survival in patients with LN (9, 29). Nevertheless, a meta-analysis revealed no association between LN and anti-P (10). Tumor necrosis factor α, or TNF-α, is responsible for co-stimulating T cells and is involved in B cell antibody production as well as B cell proliferation, differentiation, and activation. TNF-α plays a part in immunological dysfunction, tissue inflammation, and organ damage mediation (11). TNF-α blockage may result in medication-induced lupus and the production of autoantibodies (12, 28). SNPs in the TNF-α gene at position -308 are associated with an increased risk of SLE (13). In this study, we investigate whether the development of Chronic Kidney Disease in individuals with SLE is correlated with the presence of Anti-Ribosomal P Protein, ANA antibodies, and TNF-a (rs 1800629) polymorphism.

MATERIALS AND METHODS

Patients

A case-control research was undertaken on a sample of 89 patients diagnosed with systemic lupus erythematosus (SLE) aged between 10 and 64 years from September 16, 2023, to March 16, 2024. The study was done at the Nephology and Rheumatology centers in Al-Sadr Medical City, Al-Najaf. The sample consisted of 81 females and 8 men. The participants were categorized into three distinct groups based on the following:

1. Patients of SLE with LN who have chronic kidney disease.
2. Patients of SLE with LN who have no chronic kidney disease.
3. Patients of SLE who have neither LN nor CKD serve as a control group.

All age groups, males and females of Iraqi nationality, who have been diagnosed with SLE according to the established SLE criteria and who have given permission to participate in this research are eligible to be recruited. At least four of the eleven ACR factors needed to identify SLE were made by nephrologists and rheumatologists. Patients of lupus nephritis were biopsy-proven and they assessed their physical examination and clinical data over a minimum follow-up time of 6 months.

Nephrologists, identify patients who have chronic kidney disease according to their clinical and biochemical findings, GFR < 60 mL/min/1.73 m^2 for ≥3 months was the definition of chronic kidney disease.

On the opposite hand, the criteria for chosen controls were that the patients must not have symptoms or have a history of different autoimmune diseases. Furthermore, the controls should correspond to the SLE-LN patient's groups for age and ethnicity. The research was approved by the Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Faculty of Medicine, Medical Research and Ethics Committee.

Blood collection

The blood samples were obtained from patients diagnosed with Systemic Lupus Erythematosus (SLE) at the Nephrology and Rheumatology Centers, located in Al-Sadr Hospital, Najaf. From a total volume of 5 milliliters, 2 milliliters were transferred into an EDTA tube for DNA extraction to identify the TNF-a rs1800629 G/A polymorphism. The remaining 3 ml of blood was transferred into a gel tube and allowed to clot for 20 minutes at room temperature. The blood was subjected to centrifugation at 3000 RPM for 4-5 minutes. The resulting serum, free from sediment, was immediately transferred to Eppendorf tubes and preserved until it was required for the detection of immunological biomarkers.

Antibody measurement

Enzyme-linked immunosorbent assay (ELISA) (BT-LAB, China) was used to determine the serum level of ANA and Anti-Rib P antibodies. Briefly, the experiment involved preparing reagents, standard solutions, and samples at room temperature. Strips were inserted into frames, a blank well was set, and controls were added to their respective wells. Samples were incubated, washed, and HRP was added before another incubation. Substrate solutions were applied, incubated, and a stop solution was added to change the color. The optical density of each well was measured using a microplate reader set to 450 nm. The entire process was conducted with precision and proficiency to ensure accurate results.

Genotyping

PCR amplification

Throughout all SLE patient groups, genomic DNA was extracted from white blood cells (WBCs) using the Favorgen DNA extraction kit. The samples' DNA concentration was determined using a spectrophotometer (Nanodrop). The current research used primers as listed in table (1). To identify any polymorphism involving position -308 of TNF-α, TNF-α was amplified using allele-specific PCR (AS-PCR) technology.

Table 1: TNF-α (G-308A) Primers Sequence

Locus	Antisense Primers	Sense Primers	Product Size
TNF-a (G-308A)	5′-TCT CGG TTT CTT CTC CAT CG-3′	5′-ATA GGT TTT GAG GGG CAT GG-3′	**184 bp**
		5′-AAT AGG TTT TGA GGG GCA TGA-3′	

The annealing temperature for the PCR conditions was set at 65ºC for 30 seconds. The amplified product was separated for each sample on a 1.5% agarose gel, labeled with ethidium bromide, and captured on camera.

Statistical analysis

Statistical analysis of data was conducted using the statistical program GenStat, version 12, where the design two-way Analysis of Variance (ANOVA) was used to test the general significant difference between the coefficients under study. At the same time the least significant differences (L.S.D.) test was used at the probability level of 0.05 to test the significance difference between the pairwise of

the studied traits and determine which of the studied coefficients is more influential. The mean and standard deviation were used for data that were normally distributed.

RESULTS

Eighty-nine (89) patients were enrolled in this study, of which 81 (90%) were female. The mean of the age was 30.7 ± 10.7 years old. The LN was developed in 36 (40%) patients, while CKD appeared in 22 (24%). Overall CKD cases fall into LN patients, therefore the first two groups were separated into two LN groups, those with CKD and the other without CKD while the third group was SLE patients that neither LN nor CKD developed yet.

Table 1 shows the demographic and immunological status of the three groups. The prevalence of Anti-Rib-P positivity was (13.63%, 14.28%, and 3.77%) in the three groups, respectively (LSD =0.857, alpha= 0.05). The difference in Anti-Rib-P positivity between CKD-LN and non-CKD LN groups was (0.65 %) which is less than the value of the LSD indicating that no significant difference was between them. The difference between CKD-LN and SLE groups was (9.86%) greater than the LSD value indicating a high significance between these groups. The difference between non-CKD LN and SLE groups was (10.51%) which is greater than the LSD value which also indicates a high significance between these two groups. There is a strong negative linear relationship of anti-Rib-P among the three groups (r = - 0.8108) this indicates that the increase of anti-Rib-P in one group tends to decrease in the other group (Figure 1).

Table 2: Demographic and Immunological characteristics

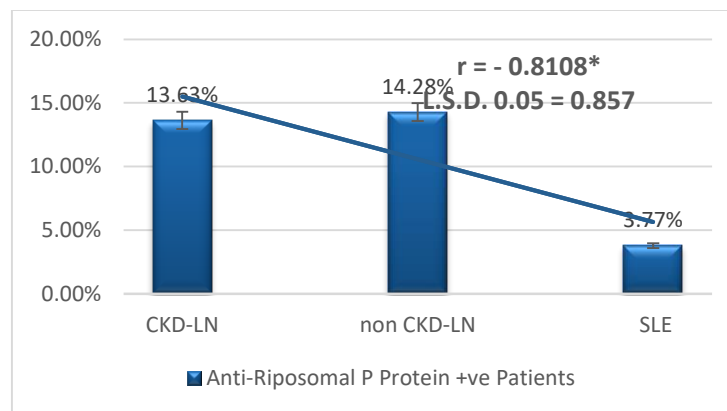| Characteristic	CKD-LN group (n=22)	Non-CKD LN group (n=13)	SLE group (n=53)	LSD value
Age, years [a]	35.9 (±11.8)	28.5(±14.3)	28.7(±8)	5.216
Female, %	86.36	92.8	92.45	4.715
Creatinine [a] (mg/dl)	3.03(±3.1)	0.72(±0.38)	0.59(±0.12)	
Anti-Rib-P(+), %	13.63	14.28	3.77	0.857
ANA(+), %	86.36	78.57	41.51	4.195

[a] means ± standard deviation

Figure 1: The correlation of Anti-Rib-P positivity among patient groups

On the other hand, the ANA positivity was (86.36%, 78.57%, and 41.51%) in the three groups respectively, (LSD=4.195, alpha=0.05). The difference between CKD-LN and non-CKD LN in ANA positivity was (7.79) while the difference between CKD-LN and SLE groups was (44.85) which is greater than the LSD value showing a high significance difference. The difference comparison between non-CKD LN and SLE groups was (37.06), which also showed a high significance between them. Also, a strong negative linear relationship of ANA among the three groups (r = - 0.9557) (Figure 2).
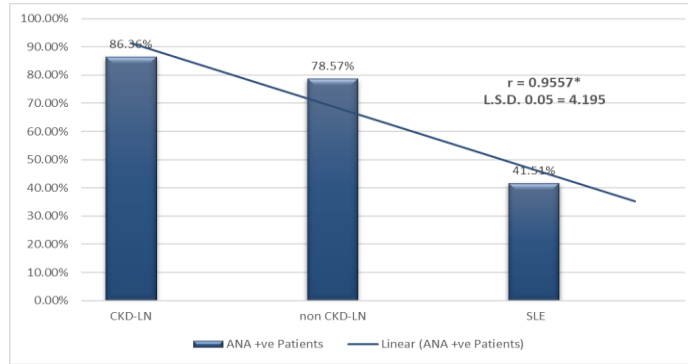
Figure 2: The correlation of ANA among patient groups

In terms of clinical manifestations, all of the proteinuria, urine cast, malar rash, and skin rash in CKD-LN were (59.09%, 13.63%, 18.18%, and 9.09%) respectively, which are higher than those in non-CKD LN (0 %. 7.14%, 14.28%, and 7.14%) while hematuria, pyuria, Joint pain, and anemia in CKD-LN were (40.90%, 45.45%, 45.45%, and 40.09%) respectively, lower than those in non-CKD LN (42.85%, 50%, 50%, and 42.85%) respectively.

Comparison between CKD-LN and SLE groups shows that proteinuria, urine cast, hematuria, pyuria, malar rash, and anemia were (59.09%, 13.63%, 40.90%, 45.45%, 18.18%, 40.09% vs. 0%, 0%, 13.33%, 13.33%, 13.33%, 26.66%) respectively, which are higher in CKD-LN than SLE group. While, joint pain and skin rash were lower in CKD-LN than in SLE (45.45%, 9.09% vs. 60%, 26.66%). The same comparison is between non-CKD LN and SLE (Figure 3).
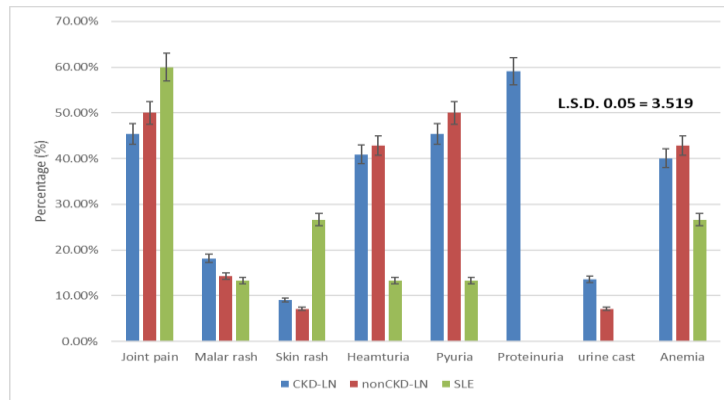

Figure 3: The correlation of clinical manifestations among groups

The study of TNF-a polymorphism was detected by Allele-Specific PCR (AS-PCR).
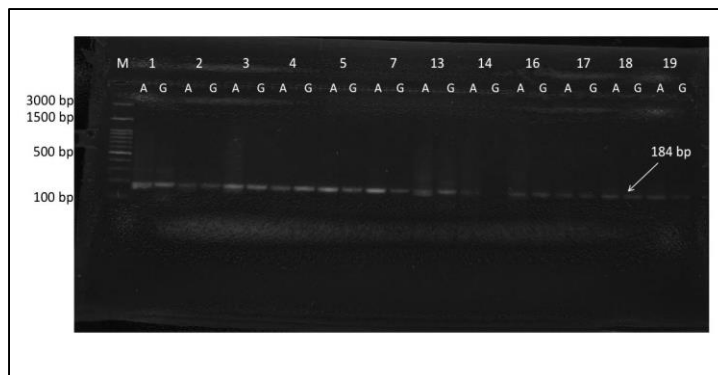

Figure 4: Electrophoresis of TNF-a G308A, M: Marker, G: Guanine, A: Adenine, bp: base pair

The distribution of TNF-a G308A revealed three genotypes at this locus: GA, GG, and AA with a band size of 184 bp for the G allele and A allele in patient and control groups (Figure 4).

AA genotype was the low frequency (13.33%) present in the CKD-LN group and disappeared in the other patient and control groups. GA genotype was the most commonly present in CKD-LN (86.66%), non-CKD LN (100%), SLE (83.33%), and the health group (100%). At the same time, the GG genotype presents in the SLE group (16.66%) only and disappears in the other groups. The allele frequency shows that the A allele is more frequent in the CKD-LN group, the G allele is more frequent in the SLE group (56.66%), and equal frequencies in non-CKD LN and healthy groups (50%). These differences were highly significant according to the Least Significant Difference (LSD = 4.920) (table 3).

Table 3: The genotype distribution of TNF-α (G-308A) gene polymorphism for patients and control

TNF-a Polymorphism		CKD-LN	non-CKD LN	SLE	Healthy
		Freq.(%)	Freq.(%)	Freq.(%)	Freq.(%)
TNF-a Genotype	GG	0 (0%)	0 (0%)	5 (16.66%)	0 (0%)
	GA	26 (86.66%)	29 (100%)	25 (83.33%)	15(100%)
	AA	4 (13.33%)	0 (0%)	0 (0%)	0 (0%)
Alleles	G	26(43.33%)	29(50%)	35(58.33%)	15(50%)
	A	34(56.66%)	29(50%)	25(41.66%)	15(50%)
L.S.D. 0.05 = 4.920					

DISCUSSION

Chronic kidney disease (CKD) is a significant factor that increases the risk of illness and death in patients with systemic lupus erythematosus (SLE). This emphasizes the importance of promptly diagnosing and treating lupus nephritis before progressing to CKD (14).

About the relationship of Anti-Rib-P and SLE, Choi et. al. performed a comprehensive review and meta-analysis on the effectiveness of anti-Rib-P as a biomarker in SLE. The study comprised over 60 papers on Neuropsychiatric SLE (NPSLE), Lupus Hepatitis (LH), Lupus Nephritis, and disease activity. Anti-Rib-P was associated with NPSLE, LN, and LH, as well as higher SLEDAI scores (15). In contrast, Shi et al. in their meta-analysis examined 16 cohort studies and showed that anti-Rib-P was linked to hepatitis and NPSLE but not renal disease (10). Nevertheless, this study showed a high prevalence of Anti-Rib-P in LN patients.

While there is limited research on the correlation between anti-Rib-P status and CKD, various studies have examined the link between anti-Rib-P and kidney outcomes in patients with LN. However, Wakamatsu et. al. showed that there is no correlation between Anti-Rib-P and CKD. While they revealed that proliferative nephritis and extended hypocomplementemia were frequently observed in five patients with both anti-P and anti-dsDNA antibodies, the incidence of CKD was not different from that of the other patients (16). Similarly, Sarfaraz et al. discovered that patients with anti-Rib-P had better renal function than those with anti-dsDNA alone, indicating that anti-P serves as a shield to protect renal outcomes (17). Furthermore, Kang et. al. showed that anti-Rib-P plays a protective function in the progression of chronic kidney disease. They reported that a single patient (3.6%) of anti-P-positive patients, showed progression to CKD throughout a follow-up period of (40.6 ± 25.9) months. On the other hand, 13 patients (25.5%) of anti-P-negative patients, developed CKD over a (45.9 ± 21.7) month period (8). These results agree with our research, which indicates that anti-P may have a protective effect on the onset of chronic renal disease. We found that (27.9%) of LN patients are anti-Rib-P positive of which (13.63%) are CKD patients while only (4%) of SLE (non-LN) patients were anti-Rib-P positive. There is a strong negative linear relationship of anti-Rib-P among

the three groups (r = - 0.8108) this indicates that the increase of anti-Rib-P in one group tends to decrease in the other group.

Several studies reported that the presence of ANA is necessary in the diagnosis of SLE. Petri et. al. reported that the new SLICC classification criteria now consider biopsy-confirmed nephritis, which is consistent with SLE according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Classification of Lupus Nephritis. This classification is sufficient when ANA or anti-dsDNA antibodies are present (18).

In addition, Kudose et. al. in their study on 300 LN patients and 560 non-LN patients, explained that ANA plays a vital role in the diagnosis and categorizing SLE and their presence along with other immunological criteria is necessary for LN diagnosis (19). The results in the present study were compatible with the previous studies where high frequencies of ANA were in the LN groups. In another study, increased levels of anti-C1q and IL-12 are associated with high percentages of ANA in Lupus Nephritis, this suggests that these biomarkers could serve as useful biological indicators for measuring disease activity and renal involvement (20).

Furthermore, Novianti et al. studied 89 pediatric SLE patients who had just been diagnosed. They found no significant relationship between the ANA pattern and renal dysfunction, as demonstrated by proteinuria (21). Similarly, In a case report study, Baksh et al. describe a patient who had repeated negative ANA titers but was given an SLE diagnosis because a kidney biopsy revealed lupus nephritis (22).

In contrast, Zeng et. al. demonstrated that ANA levels increase in cases of renal failure. According to their research, the levels of each ANA IgG subclass were considerably greater in the active instances of SLE and LN groups compared to the inactive groups (23). This finding is in line with previous studies. Also, Hemdan and Alsayed declared that the ANA profile (IgG) can serve as an indicator of severe kidney damage and activity in patients with SLE and LN (24). In our study, we found that ANA is more significant in the CKD-LN group than in the other groups. In addition, there is a strong negative relationship among the groups (r = - 0.9557) which indicates the increase of ANA in one group can be decreased in the other. So, ANA antibodies in LN patients may be an indicator of CKD.

About TNF-a polymorphism, in a meta-analysis, Sadia found a correlation between the TNF-α - 308G/A variant and a higher likelihood of developing several autoimmune disorders. This suggests that the A allele of the TNF-α gene plays a role in the underlying mechanisms of various illnesses. The author also showed a strong correlation between the TNF-α-308G/A gene polymorphism and an elevated risk of numerous autoimmune diseases. This link was detected in both the allelic (A vs G) and heterozygous genotypes (AG vs GG) among Asian populations (25). Ranganath et. al. show that TNF-a (G308A) gene polymorphism are implicated in End Stage Renal Disease (ESRD) and AA of TNF-a 2G308A and 2G238A genes were high-risk genotypes as the risk was increased (6.47) and (3.3) fold when they included the risk genotypes in ESRD cases and controls (26)(27). Our results are similar, we found that the AA genotype presents only in CKD-LN and the A allele presents more frequently (56.66 %) than in other groups. We suggest that TNF-a (G308A) gene polymorphism may be associated with a higher likelihood of developing CKD in SLE patients specifically LN ones.

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