AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Evaluation of Anti-ds-DNA and Anti-Smith Autoantibodies in Systemic Lupus Erythematosus Patients Associated with Chronic Kidney Disease.

Hadi Abdulridha Hadi [1], Dheyaa Shnan Abdulkadhim Al-Jameel [2], Alaa Abdlhussein Abdulzahra Alshibly [3]

[1,2,3] *Department of Medical Microbiology, Faculty of Medicine, Jabir ibn Hayyan University of Medical and Pharmaceutical Sciences. Najaf, Iraq.*

Article history
Received: 6/6/2024
Revised: 24/7/2024
Accepted: 8/8/2024

*Corresponding Author: *Hadi Abdulridha Hadi, Department of Medical Microbiology, Faculty of Medicine, Jabir ibn Hayyan University of Medical and Pharmaceutical Sciences. Najaf, Iraq*;
Email: pstm.hadi.a.hadi@jmu.edu.iq
DOI: 10.36320/ajb/v16.i3.16495

Abstract: Background: 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). Objectives: This study aimed to investigate if there is a correlation between CKD development in patients of SLE and the presence of anti-smith and anti-ds-DNA biomarkers. Materials and Methods: A case-control study of 89 SLE patients. They measured anti-Sm and anti-ds-DNA antibodies by Enzyme-Linked Immunosorbent Assay (ELISA). Results: Eighty-nine (89) patients were enrolled in this study, The LN was developed in 36 (40%) patients. The average of anti-Sm positivity was 14.28 % in non-CKD LN more than in CKD-LN (9.09%) and SLE (3.77%) groups (LSD=2.067). Anti-ds-DNA positivity was (28.57%) in non-CKD LN, (9.09%) in the CKD-LN group, and (5.66%) in the SLE group (LSD=4.142). They were 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, alpha = 0.05). Conclusion: This study discovered that individuals with non-CKD lupus nephritis had notably elevated levels of anti-Sm and anti-dsDNA antibodies compared to patients with CKD-related lupus nephritis and SLE patients without kidney involvement. This suggests that these antibodies may serve as characteristic markers for this particular subset of individuals with lupus nephritis. Moreover, their existence may have an impact on the early phases of the illness, perhaps leading to the development of chronic renal disease in the future.

Keywords: Systemic Lupus Erythematosus, Chronic Kidney Disease, Lupus Nephritis, Anti-Smith, Anti-dsDNA

1. Introduction

Systemic lupus erythematosus (SLE), sometimes known as lupus, is a complicated autoimmune disease characterized by a chronic course marked by relapses and flare-ups. It has many symptoms which may range from moderate to life-threatening conditions. Genetic susceptibility, environmental, immunological, and hormonal variables interrelate to cause the clinical development of SLE, with a robust prevalence for women of reproductive age [1]. Clinical manifestations in SLE are numerous and can affect every part, the most frequent significant complication of SLE is lupus nephritis (LN), which affects over half of its patients and can lead to end-stage renal disease (ESRD) which is the most substantial and expensive consequence [2].

Patients with lupus continue to encounter a significant risk of morbidity and mortality regardless of recent advancements in the treatment of the condition. For instance, about 10% of people with lupus nephritis (LN) progress to develop end-stage renal disease [3], [4]. One study displays a considerable correlation between renal flare and the progress of chronic kidney disease (CKD), as well as a predisposition between renal flare and the progression of CKD in individuals with LN [5].

Anti-dsDNA antibody positivity rates in the LN group are often greater than those in the non-LN SLE group and the anti-dsDNA is one of the risk factors for SLE complicated with LN [6].

DOI: 10.36320/ajb/v16.i3.16495

Anti-smith antibody is associated with disease activity and clinical manifestation, in a cohort study, numerous clinical symptoms, including renal, neurologic, hematologic problems, and vasculitis, have been linked to the presence of anti-Sm antibodies [7].
This study aimed to investigate if there is a correlation between development of CKD in patients of SLE along with the presence of anti-smith and anti-dsDNA biomarkers.

2. Methodology

Patients Enrollment

A case-control study was conducted from September 16, 2023, to March 16, 2024, on a sample of 89 patients with systemic lupus erythematosus (SLE), ages 10 to 64. The study was conducted at the Al-Sadr Medical City, Al-Najaf nephrology and rheumatology centers. There were eight men and eighty-two women in the sample. Based on the following, the participants were divided into three different groups:

1. SLE patients with chronic renal disease who possess LN.

2. SLE patients without chronic renal disease but possess LN.

3. SLE patients who have neither CKD nor LN, represent the control group.

Inclusion and Exclusion Criteria

All individuals of Iraqi nationality, regardless of age, who have been diagnosed with SLE based on the standard criteria for SLE and have provided permission to participate in this study, are eligible for recruitment. Nephrologists and rheumatologists determined at least four out of the eleven ACR criteria required to diagnose SLE, the last update version EULAR/ACR 2019 was taken in consideration to diagnose the SLE patients. Lupus nephritis patients underwent biopsy-based diagnosis beside physicians' assessment according to the clinical examination and laboratory investigations. They evaluated based on their physical examination and clinical data. Chronic kidney disease patients were diagnosed by nephrologists based on clinical and biochemical data, with a GFR of < 60 mL/min/1.73 m2 or persistence of proteinuria (>500 mg/day) for ≥3 months. On the other hand, the criteria for selecting controls were that the patients had no symptoms or a history of any other autoimmune disease. In addition, the controls should match the age and ethnicity categories of the SLE-LN patients.

Patients with other autoimmune diseases, patients with cancer or a history of malignancies, patients with diabetes mellitus, patients with urinary tract infections, patients with kidney transplantation, and patients with incomplete medical documentation to confirm the diagnosis of SLE and CKD.

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. A volume of 5 milliliters 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 Anti-ds-DNA and Anti-Smith Autoantibodies. 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.

.3. Results

Baseline Characteristics

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.

DOI: 10.36320/ajb/v16.i3.16495

Demographic and Immunological Profiles

Table 1 shows the demographic and immunological status of the three groups. The proportion of Anti-Sm positivity was (9.09%, 14.28%, and 3.77%) in the three groups respectively, (LSD =2.067, alpha= 0.05). The difference in proportions between CKD-LN and non-CKD LN groups was (5.19%) which is more than the value of the Least Significant Difference (LSD=2.067) indicating a highly significant difference between them. Similarly, the difference was highly significant between non-CKD LN and SLE as the difference in proportion was (5.09%) and a highly significant difference was also between CKD-LN and SLE groups where the proportion difference was (5.32%). Both differences are more than the LSD value (2.067) indicating a highly significant difference. There is no correlation among patient groups regarding these markers as demonstrated by Pearson's test (r=0.2562) as shown in (Figure 1).

On the other hand, the Anti-ds-DNA proportions were (9.09%, 28.57%, and 5.66%) in the three groups respectively, (LSD=4.926, alpha=0.05). The proportion difference between CKD-LN and non-CKD LN was (19.48) which is greater than the LSD value showing a high significance difference. In contrast, the difference between CKD-LN and SLE groups was (3.43) which is less than the LSD value showing no significant difference. The proportion comparison between non-CKD LN and SLE groups was (22.91), which showed a high significance between these two groups (Figure 2).

Treatments Used Comparison in Patient Groups

All the patients were on a treatment regimen. So, after comparing treatment strategies, we found a significant difference among groups (LSD = 6.285, alpha= 0.05).

Mycophenolate mofetil, Mycophenolic acid, and Prednisolone are used more highly in CKD-LN (36.3%, 68.2%, and 50%) than non-CKD (28.5%, 57.1%, and 35.7) and SLE (22.6%, 62.2%, and 35.8%) respectively. A highly significant difference was between CKD-LN and non-CKD LN for the drugs as the average absolute difference between Mycophenolate mofetil in both groups was (7.5%), Mycophenolic acid (11.1%), and Prednisolone (14.3%) which were greater than the LSD value (LSD=6.285). While, a highly significant difference was between the CKD-LN group and the SLE group in Mycophenolate mofetil and Prednisolone but no significant difference in Mycophenolic acid, the absolute differences for Mycophenolate mofetil, Prednisolone, and Mycophenolic acid were (13.7%, 14.2%, and 6%) respectively.

Tacrolimus, Azathioprine, and Rituximab are used more highly in the non-CKD LN (28.8%, 14.3%, and 7.1%) than in the CKD-LN (13.6%, 13.6 %, and 0 %) and SLE (13.2%, 7.5%, and 0%) respectively. The absolute difference among drugs between non-CKD and CKD LN groups was for Tacrolimus (15.2%), Azathioprine (0.7%), and Rituximab (7.1%) which indicated a highly significant difference in Tacrolimus and Rituximab as the average is greater than the LSD value (6.285). The comparison between non-CKD LN and SLE groups in average difference was for Tacrolimus (15.6%), Azathioprine (6.8%), and Rituximab (7.1%) indicated a highly significant difference in the three drugs.

The last two drugs, Hydroxychloroquine and Methotrexate, were higher in the SLE group than the LN two groups. Still, they had no significant differences as the absolute differences were lower than the LSD value (Figure 3).

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-Sm (+), %	9.09	14.28	3.77	2.067
Anti-ds-DNA(+), %	9.09	28.57	5.66	4.142

Table 1: Demographic and Immunological characteristics (a means ± standard deviation)

DOI: 10.36320/ajb/v16.i3.16495

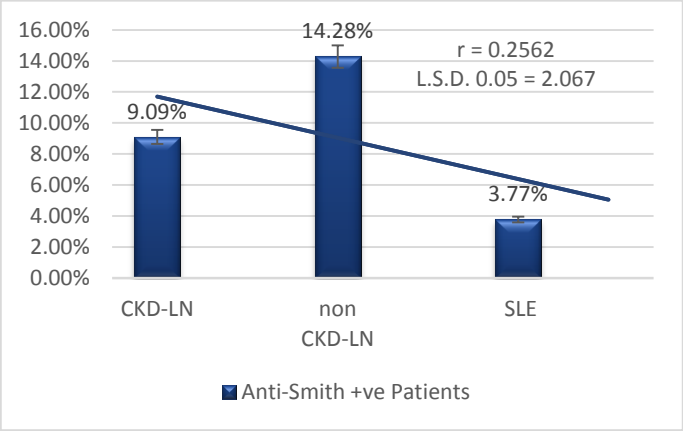

Fig. 1: The correlation of Anti-Sm among patient groups.

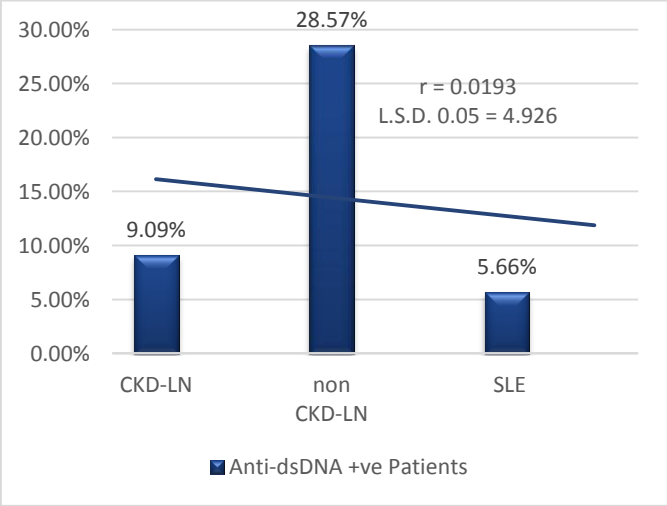

Fig. 2: The correlation of Anti-ds-DNA among patient groups.

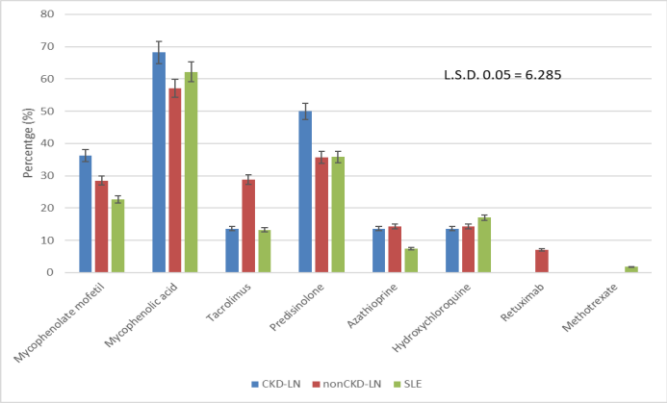

Fig. 3: Comparison of treatment use among patient groups.

4. Discussion

Chronic Kidney Disease (CKD) is an important factor contributing to increased susceptibility to the illness and mortality in patients with SLE. This underlines the significance of immediately identifying and dealing with LN before it develops into CKD [8].

While there is limited research on the correlation between Anti-Sm and CKD, Ahn et. al. propose that the presence of anti-Sm antibodies detected during LN patients may serve as an indicator of early poor outcomes in the first stages of follow-up. Furthermore, they discovered that the presence of anti-Sm antibodies was autonomously linked to the requirement of immuno-suppressants to maintain the stability of lupus nephritis [9].

In a case sectional study into the correlation between anti-Sm and clinical manifestations, Arroyo-Avila et al. discovered that anti-Sm antibodies were strongly linked to renal involvement, however, CKD is not specifically stated [7]. In contrast, a study conducted on 201 Puerto Rican patients found connections between the presence of anti-Sm antibodies and several kidney-related conditions such as proteinuria, hematuria, urinary cellular casts, nephrotic syndrome, insufficient renal function, and chronic kidney disease [10]. However, in our study, we found that higher levels of anti-Sm antibodies were associated with the LN groups and there was no significant difference in the CKD-LN group.

Regarding anti-ds-DNA, Somnath et. al. stated that the significance of the case series lies in the absence of kidney-related symptoms in SLE patients who are negative for anti-dsDNA antibodies. The researchers found a correlation between renal symptoms and the presence of anti-ds-DNA antibodies [11]. Similarly, in another study, showed that Patients who had a considerable increase in anti-ds DNA antibodies at the time of the study were more likely to have renal illness than patients who did not have such an increase [12].

We also found that anti-ds-DNA antibodies are more related to the LN than non-renal SLE patients. Gensous et. al. in a systematic review observed varied outcomes in terms of sensitivity ranging from 27-100% and specificity ranging from 13-89% of anti-dsDNA tests as indicators of LN, yielding unsatisfying results [13]. This is in line with our study, we also found fluctuation in the anti-ds-DNA levels. However, drugs are also a concern, a problem in many studies was the failure to distinguish between incident and prevalent patients, as well as the lack of clear reporting on the specific therapy employed.

This is an important point because potent immunosuppressive drugs, which are commonly used in severe cases of LN, can likely decrease antibody levels [13]. The same limitation was in our study, all the patients were on a treatment course, which may affect and reduce the levels of anti-ds-DNA.

In a study conducted in Brazil, Briele Keiserman et. al. found that the simultaneous presence of IgM anti-dsDNA may have a protective effect on kidneys. Furthermore, this could explain why, in medical practice, some SLE patients with a positive IgG anti-ds-DNA test do not display any kidney complications, as the IgM isotype is not regularly examined [14]. In this study, significant patients with LN did not develop kidney damage represented by CKD. As was already demonstrated anti-ds-DNA could be protective against certain types of tissue injury. Target tissue dsDNA deposition has the potential to trigger an inflammatory cascade that results in tissue damage. Such damage may be avoided or inverted if anti-ds-DNA antibodies interfere with this cascade. Individualizing medicine based on patient-specific biological indicators may thus become practicable [15].

Conclusion

The non-CKD LN group had a considerably higher prevalence of anti-Sm and anti-dsDNA antibodies than the CKD-LN and SLE groups. This finding implies a possible differentiating feature among these groups of patients. This could emphasize the potential significance of these markers in distinguishing between various subtypes of LN. Moreover, these antibodies could have a function in the early stages of the illness, possibly leading to the development of chronic kidney disease (CKD).

Acknowledgment

We would like to thank the Department of medical Microbiology at the University of Jabir Ibn Hayyan of Medical and Pharmaceutical Sciences for providing the necessary facilities. We are grateful to the staff in Nephrology and Rheumatology centers of Al-sadr hospital for their assistance during samples collection period**.**

Ethics

The Medical Research and Ethics Committee of the Faculty of Medicine, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences authorized the study.

References

1. Basta, F., Fasola, F., Triantafyllias, K., & Schwarting, A. (2020). Systemic lupus erythematosus (SLE) therapy: The old and the new. Rheumatology and Therapy, 7(3), 433–446. doi: Not provided

2. Tektonidou, M. G., Dasgupta, A., & Ward, M. M. (2016). Risk of End-Stage Renal Disease in Patients with Lupus Nephritis, 1971-2015: A Systematic Review and Bayesian Meta-Analysis. Arthritis Rheumatology, 68(6), 1432–1441. doi: 10.1002/art.39594

3. Borchers, A. T., Keen, C. L., Shoenfeld, Y., & Gershwin, M. E. (2004). Surviving the butterfly and the wolf: Mortality trends in systemic lupus erythematosus. Autoimmunity Reviews, 3(6), 423–453. doi: 10.1016/j.autrev.2004.04.002

4. Frodlund, M., Reid, S., Wettero, J., Dahlstrom, O., Sjowall, C., & Leonard, D. (2019). The majority of Swedish systemic lupus erythematosus patients are still affected by irreversible organ impairment: Factors related to damage accrual in two regional cohorts. Lupus, 28(10), 1261–1272.

5. Parikh, S. V., Nagaraja, H. N., Hebert, L., & Rovin, B. H. (2014). Renal flare as a predictor of incident and progressive CKD in patients with lupus nephritis. Clinical Journal of the American Society of Nephrology, 9(2), 279–284.

6. Yin, F., Pang, C., Lyu, F., Zhang, W., Zhao, J., & Wang, Y. (2017).

Significance of anti-nucleosome antibody, anti-C1q antibody and anti-double stranded DNA antibody in diagnosis of lupus nephritis. Journal of Jilin University (Medicine Ed.), 757–761.

7. Arroyo-Ávila, M., et al. (2015). Clinical associations of anti-Smith antibodies in PROFILE: A multi-ethnic lupus cohort. Clinical Rheumatology, 34, 1217–1223.

8. Mageau, A., et al. (2019). The burden of chronic kidney disease in systemic lupus erythematosus: A nationwide epidemiologic study. Autoimmunity Reviews, 18(7), 733–737.

9. Ahn, S. S., Yoo, B., Song, J. J., Park, Y., Lee, S., & Lee, S. (2016). Anti- Sm is associated with the early poor outcome of lupus nephritis. International Journal Rheumatology Diseases, 19(9), 897–902.

10. Vilá, L. M., Molina, M. J., Mayor, A. M., Peredo, R. A., Santaella, M. L., & Vilá, S. (2006). Clinical and prognostic value of autoantibodies in Puerto Ricans with systemic lupus erythematosus. Lupus, 15(12), 892–898.

11. Maitra, S., Sarkar, S., Mukherjee, B., & Ghosh, S. (2022). Variable presentations of systemic lupus erythematosus based on presence or absence of antidouble stranded DNA antibodies: A case series. Asian Journal of Medical Sciences, 1, 175–179. doi: 10.3126/ajms.v13i1.40369

12. Ragunathan, L., Kannaiyan, A. C., & N, C. (2020). Immunodiagnosis of Systemic Lupus Erythematosus (SLE) in a Tertiary Care Hospital. Annals of Pathology and Laboratory Medicine, 7(4). doi: 10.21276/APALM.2718

13. Gensous, N., et al. (2017). Predictive biological markers of systemic lupus erythematosus flares: A systematic literature review. Arthritis Research & Therapy, 19(1-12), 238. doi: 10.1186/s13075-017-1442-6

14. Keiserman, B., Ronchetti, M. R., Monticielo, O. A., Keiserman, M. W., & Staub, H. L. (2013). Concomitance of IgM and IgG anti-dsDNA Antibodies Does Not Appear to Associate to Active Lupus Nephritis. Open Rheumatology Journal, 7(1), 101–104. doi: 10.2174/1874312901307010101

15. Schiffer, L. E., et al. (2002). Lowering anti-dsDNA antibodies—what's new. Lupus, 11(12), 885–894.