Original Article

Prevalence of Serum Cryoglobulins in Patients with Proliferative Lupus Nephritis and its Relation to Srological Markers of Disease Activity

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Abstract

Background: Cryoglobulins are found in patients with systemic lupus erythematosus (SLE) with a variable percentage but their relation to disease activity in patients with proliferative lupus nephritis is not determined yet. **Objectives:** To determine the prevalence of cryoglobulinemia in patients with proliferative lupus nephritis and its relation to disease activity. **Methods:** In a comparative cross-sectional study we investigated 60 candidates, 30 patients with renal biopsy proven proliferative Lupus nephritis (LN), 15 patients with SLE without nephritis and 15 healthy subjects. Serum samples were obtained at 37°C, and cryoglobulinemia was estimated by centrifugation at 4°C after incubation for 7 days in all the studied groups. We also compared between cryo-positive patients and cryo-negative patients according to serological markers of disease activity and activity and chronicity indices in renal biopsy. **Results:** Cryoglobulins were detected in the sera of 2 patients in the LN group (6.7%) , 1 patient in the SLE without nephritis group (6.7%) and non of the healthy subjects. Rheumatoid factor (RF) titre was significantly higher in cryo-positive patients (p=0.006). Complement levels; C3 and C4 were significantly lower in cryo-positive patients (p=0.006 and 0.011 respectively). There was no statistically significant difference between cryo-postive and cryo-negative patients as regard Anti-double stranded (ds DNA) titre, activity or chronicity indices in renal biopsy. **Conclusion:** Cyoglobulins were positive in 6.7% of cases in our study with no difference between patients with or without nephritis and their presence were not related to pathological markers of disease activity in patients with proliferative LN.

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INTRODUCTION

Among patients with lupus, LN affects both sexes equally, is more severe in children and men, and is less so in older adults. The incidence of LN is about 30% in White, 60% in Black and Hispanic, and 40% to 80% in Asian patients with SLE. Nephritis is more common in African Americans and Hispanics than in whites, and is also more in men than in women. Renal damage is more likely to develop in nonwhite groups.[1-4]

Patients with International Society of Nephrology (ISN) class I biopsies usually have no evidence of clinical renal disease. Similarly, patients with ISN class II may have elevated anti-dsDNA or low complement levels, dut usually they do not have active urinary sediment, hypertension is uncommon, the GFR is normal, and proteinuria is rarely above 1 gram/24 hours. Patients with class I and class II LN in renal biopsy have a good renal prognosis unless they transform to another class.[5]

Patients with active ISN class IIIA or IIIA/C usually have microscopic hematuria, hypertension, low complement levels, and proteinuria.[5] Patients with mild proliferation affecting a few glomeruli usually have good response to treatment, with less than 5% progressing to renal failure during 5 years of follow-up. Others with more glomerular affection or with necrotizing lesions and crescent formation have a prognosis similar to that of class IVA patients.[6]

Patients with ISN class IVA usually have high serologic activity (low serum complement and high anti-dsDNA–binding activity). Clinically, they have active urinary sediment, hypertension, heavy proteinuria, and reduced GFR. Class IV diffuse proliferative disease has the worst renal prognosis in all studies. [7]

Cryoglobulinemia is a clinical disorder characterized by the presence of cryoglobulins in the serum. Cryoglobulins are immunoglobulins that have a specific physical feature of being able to precipitate at cold temperatures and re-dissolve when rewarmed. Cryoglobulinemia may be asymptomatic without end-organ damage and is sometimes accidentally discovered. In type I cryoglobulinemia, the cryoglobulins are monoclonal immunoglobulins (Ig's), usually of the IgG or IgM isotypes and rarely IgA or free immunoglobulin light chains.[8] Type I cryoglobulinemia develops in the case of protein-secreting monoclonal gammopathies.[9,10]
In type II cryoglobulinemia, the cryoglobulins are a mix of monoclonal IgM with rheumatoid factor (RF) activity and polyclonal IgG. This type is usually associated with hepatitis C virus (HCV) infection in up to 90% of patients.[11] Other causes of type II cryoglobulinemia include other infections (mainly HIV and hepatitis B virus [HBV]), connective tissue diseases (CTDs) like SLE, and lymphoproliferative disorders. Approximately 10% of patients have unknown cause (termed essential mixed cryoglobulinemia). Type III cryoglobulins are by polyclonal IgM with RF activity and polyclonal IgG. This type is seen in CTDs or secondary to infection (mainly HCV). Generally, cryoglobulins in mixed cryoglobulinemia result from a B-cell proliferation in the setting of chronic immune activation induced by chronic infection, autoimmune disease, or an unknown cause.[12] However, its role in the pathogenesis and activity of lupus nephritis hasn't been established yet.

PATIENTS & METHODS

We conducted a comparative cross sectional study in which patients were divided into three groups: Group (I): Thirty patients with biopsy proven proliferative lupus nephritis, group (II): Fifteen patients with SLE without lupus nephritis and group (III): Fifteen age and sex matched healthy subjects. We excluded patients with positive hepatitis c virus (HCV) antibodies and antiphospholipid antibodies.

All patients in the study were subjected to thorough history taking with emphasis on symptoms suggestive of cryoglobulinemia as hyperviscosity symptoms (like headache, blurring of vision), skin rash, neuropathy, (as burning sensation and numbness), urinary symptoms (as red or frothy urine) or previously diagnosed thrombotic episodes. They were also well examined for signs of cryoglobulinemia like vasculitic rash and neuropathy.

Ultrasound guided percutaneous renal biopsy with light microscopic examination of obtained specimen and estimation of activity and chronicity index was done.

Complete blood count (CBC), Serum urea & creatinine, complete urine analysis, Urinary protein/creatinine ratio, Anti-nuclear antibody (ANA), Anti dsDNA, Complement factors C3 & C4, Erythrocyte sedimentation rate (ESR) & C-reactive protein (CRP), Rheumatoid factor (RF) and serum cryoglobulins were done.

Blood samples were obtained and kept at 37°C for 30 minutes before separation. Serum was prepared by centrifuging at 37°C for 10 minutes at 2,500 rpm. Fresh centrifugated serum was incubated at 4°C for 7 days after collection and examined for cryoprecipitation.

Statistical Analysis:

We used conventional chi-square and Fisher's exact tests to analyze qualitative differences. For comparison of quantitative parameters, Student t test was used for normally distributed quantitative variables to compare between two studied groups, and the nonparametric Mann-Whitney U test was used for abnormally distributed quantitative variables to compare between two studied groups. F-test (ANOVA) was used for normally distributed

quantitative variables to compare between more than two groups and Kruskal Wallis test for abnormally distributed quantitative variables to compare between more than two studied groups. Post Hoc (Dunn's multiple comparisons test) was used for pairwise comparisons.

A value of P 0.05 indicated statistical significance. This statistical analysis was performed by the SPSS program (SPSS Inc, Chicago, IL) with the information stored in the database program.

RESULTS:

Cryoglobulins were detected in the sera of 3 patients (6.7%), of whom 1 was a female with SLE without nephritis and 2 were females with proliferative LN.

The duration of SLE ranged between 0.0 – 9.0 years with a median of 2.0 years in group I and 0.20 – 17.0 years with a median of 2.0 years in group II with no statistically significant difference (p=0.608). At the study initiation, the mean age of patients was 31.93 ± 8.68 in group I, 32.60 ± 10.08 in group II and 30.60 ± 8.25 in group III with no statistically significant difference between the three groups (p=0.821).

Table I shows the affected body systems by SLE in the three studied groups.

Table I: Comparison between the three studied groups according to the affected systems

Systems affected	Group I (n = 30)		Group II (n = 15)		Group III (n = 15)		χ^2	P
	No.	%	No.	%	No.	%		
Kidney	30	100.0	0	0.0	0	0.0	–	–
Skin	7	23.3	6	40.0	0	0.0	1.352	^{FE}p=0.304
CNS	2	6.7	2	13.3	0	0.0	0.549	^{FE}p=0.591
Blood	6	20.0	3	20.0	0	0.0	0.00	^{FE}p=1.000
Heart	0	0.0	2	13.3	0	0.0	4.186	^{FE}p=0.106
Joints	10	33.3	8	53.3	0	0.0	1.667	0.197
Serosa	2	6.7	2	13.3	0	0.0	0.549	^{FE}p=0.591
Lung	1	3.3	0	0.0	0	0.0	–	–

χ^2: **Chi square test** FE**: Fisher Exact**
p: p value for comparing between the studied groups
Group I: LN
Group II: SLE without nephritis

As shown in table II on comparing between cryo-positive and cryo-negative patients according to serological and pathological markers of the disease activity, we found that C3 had a median of 70.5 mg/dl in cryo negative patients and 26.0 mg/dl in cryo positive patients (p=0.006). C4 had a median of 10.95 mg/dl in cryo negative patients and 4.0 mg/dl in cryo positive patients (p=0.011). Anti-Ds DNA titre had a median of 134.5 IU/mL in cryo negative patients and 130.0 IU/mL in cryo positive patients (p=0.909). RF titre had a median of 8.0 IU/mL in cryo negative patients and 16.50 IU/mL in cryo positive patients (p=0.006).

Table II: relation between cryoglobulinemia and C3, C4, RF and anti-ds DNA titre

	CRYO		U	P
	Negative (n=42)	Positive (n=3)		
C3				
Min. – Max.	12.0 – 176.0	25.0 – 27.0		
Mean ± SD.	71.05 ± 31.81	26.0 ± 1.0	3.0*	0.006*
Median	70.50	26.0		
C4				
Min. – Max.	3.0 – 47.0	2.10 – 5.0		
Mean ± SD.	14.13 ± 10.60	3.70 ± 1.47	7.0*	0.011*
Median	10.95	4.0		
Anti-Ds DNA				
Min. – Max.	15.0 – 1000.0	32.0 – 173.0		
Mean ± SD.	183.26 ± 210.41	111.67 ± 72.27	60.50	0.909
Median	134.50	130.0		
RF				
Min. – Max.	3.90 – 13.30	11.50 – 24.70		
Mean ± SD.	7.66 ± 2.32	17.57 ± 6.66	2.50*	0.006*
Median	8.0	16.50		

U: Mann Whitney test
p: p value for comparing between **Negative** and **Positive**
*: Statistically significant at $p \leq 0.05$

The activity index (AI) ranged between 1.0 – 12.0/24 with a median of 7.0/24 in cryo negative patients and 6.0 – 10.0/24 with a median of 8.0/24 in cryo positive patients with no significant statistical difference between the two groups (p=0.901).

The chronicity index (CI) ranged between 0.0 – 10.0/12 with a median of 3.0/12 in cryo negative patients and 0.0 – 0.0/12 with a median of 0.0/12 in cryo-positive patients with no significant statistical difference between the two groups (p=0.074) as shown in table III.

Table III: relation between cryoglobulinemia and activity and chronicity indices in the renal bioosy

	CRYO		U	P
	Negative (n=28)	Positive (n=2)		
AI/24 (n=27)				
Min. – Max.	1.0 – 12.0	6.0 – 10.0		
Mean ± SD.	7.57 ±2.66	8.0 ±2.83	26.50	0.901
Median	7.0	8.0		
CI/12				
Min. – Max.	0.0 – 10.0	0.0 – 0.0		
Mean ± SD.	3.54 ±2.89	0.0 ±0.0	6.0	0.074
Median	3.0	0.0		

U: Mann Whitney test
p: p value for comparing between **Negative** and **Positive**
*: Statistically significant at $p \leq 0.05$

C3 and C4 were significantly lower in cryo positive patients. RF titre was significantly higher in cryo positive patients. But there was no statistically significant difference between cryo positive and cryo negative patients as regard Anti-DsDNA titre, serum creatinine, urinary protein/ creatinine ratio or activity and chronicity indices in the renal biopsy.

DISCUSSION

In this study, we have investigated 60 subjects, 30 patients with proliferative lupus nephritis, 15 patients with SLE without renal affection and 15 healthy control subjects. Females represented 95% of all cases forming 93% of the LN group and 100% of the group without nephritis. This is consistent with the female predominance shown in previous studies.

The mean age of the three study groups was around 30 years. This is consistent with the literature data that SLE occurs more commonly between 15and 45 years of age. Group I and group II had a comparable duration of the disease with a mean of 2 years in group I and 3 years in group II.

As regards the affected systems, apart from the kidney that was affected in 100% of group I and not affected at all in group II, the most common presentation of SLE was musculoskeletal affection (40% of cases), followed by mucocutaneous affection (28 % of cases),other affected systems included : blood, lungs, CNS, the heart and serosal surfaces.

In this study, we compared between patients of SLE without nephritis, patients with proliferative LN and healthy controls as regards their routine laboratory investigations, serological investigations for SLE activity and cryogolbulinemia. As ruled in the inclusion and exclusion criteria of each group in the study, all patients in group II had normal renal function tests, normal urinay protein creatinine ratio and normal urine analysis findings.

In group I, they all had renal biopsy proven proliferative LN. The most commonly found pathology is the diffuse proliferative form with or without superadded membranous affection accounting for 63% of cases.

As regards cryoglobulinemia, cryoglobulins were found in 6.7% of SLE patients either with or without LN. There was no relation between the presence of cryoglobulins and the occurrence of LN. Also there was no relation between the presence of cryoglobulins and the activity or chronicity indices in renal biopsy.

Previous studies done to investigate the prevalence of cryoglobilinemia in SLE showed different results ranging from 16% to 83% in small series of cases. A study done in Barcelona in 2001 on a large series of 122 SLE patients found that cryoglobulins were positive in 25% of cases.

In this study neither HCV positive nor antiphospholipid positive patients were excluded. They found that a cryocrit greater than 1% was more frequent in those SLE patients with HCV infection. As our study showed they found a higher frequency of some immunologic markers in cryoglobulinemic SLE patients (RF and hypocomplementemia).

The association between hypocomplementemia and cryoglobulins is well known. Adu and Williams [13] described the ability of SLE cryoglobulins to fixate complement in vitro and suggested that these immune complexes can fixate complement in vivo and so, they cause tissue damage in this disease.

Roberts et al., [14] suggested that, in SLE patients with diffuse proliferative glomerulonephritis, cryoglobulins and glomerular immune deposits can fixate complement via the classic and alternative pathways. They found a higher prevalence of RF in cryoglobulinemic SLE patients, may be due to the RF activity of some cryoglobulinemic component. RF can be used as an immunologic marker that points to the presence of cryoglobulinemia in SLE patients.[15]

A cross-sectional study in Isfahan, Iran and was conducted from September 2010 to May 2011. They studied 80 women with SLE, they found a prevalence of 48.8% of cryoglobulins in a large number of SLE patients, all of them showed high titre of circulating cryoglobulins (cryocrit >5%). There was a correlation between Anti-dsDNA, ANA, CRP, and hypocomplementemia and cryoglobulinemia in the studied SLE patients. These finding may identify the SLE patients with cryoglobulinemia. That study helped in the recognition of immunological characteristics of SLE which are involved in systemic inflammation.

In 2016 a case-control study was done. All patients with a cryoglobulinemia between January 2005 and December 2016 in a third level referral centre in Mexico City were included. Cryoglobulinemic SLE patients (cryocrit 1%) were included in the case group, whereas non cryoglobulinemic SLE patients were considered controls.

The investigators studied the demographic, clinical and immunological characteristics at the time of the positive cryoglobulin result, as well as three months earlier, and 6 and 12 months later. Thirty-six SLE patients had a positive test for cryoglobulins throughout the study period. Ten patients had cryocrit of 1% and were included in the case group, whereas 26 patients with a negative test were included as controls. Mean age was 37.7±18.3 in cases and 41.7±19.3 in controls. Women represented 70% of cases and 88.5% of controls.

Among the case group, the cryocrit was 1% in 9 patients, and 3% in one. Regarding clinical and immunological features, a positive lupus anticoagulant and vasculitis were more common in cryoglobulinemic patients (p=0.004 and 0.04, respectively). At the time of the cryoglobulin detection, patients in the case group had lower levels of C3 and C4 (p=0.026 and p=0.003, respectively), and serum albumin (p=0.028). They also had a higher frequency of serositis (p=0.021), peripheral oedema (p=0.034) and SLICC Damage Index score (p=0.014) than controls.

On follow-up, the cryoglobulinemic patients had a higher SLEDAI score after six and twelve months (p=0.009 and 0.034, respectively). After 12 months they had a higher frequency of renal activity (p=0.004) and lower C4 levels (p=0.001). Among patients with renal activity, 20% of cases and 55% of controls had achieved complete remission after 12 months. So according to that study serum cryoglobulins in SLE patients were relates to positive lupus anticoagulant and hypocomplementemia. At follow-up, patients with cryoglobulinemia had a higher frequency of renal activity, as well as an increased disease activity overall.[16]

Comparing our study to the previous studies, the prevalence of cryoglobulinemia found in our study was lower with no difference between LN and non LN patients. Cryoglobulinemia was related to disease activity in SLE represented by hypocomplementemia. There was no relation between the presence of cryoglobulins and activity or chronicity indices in the renal biopsies of LN patients.

These differences between our results and the results of the previous studies may be attributed to exclusion of HCV positive patients who represented some percentage of positive cryoglobulins in SLE patients in the previous studies.

The same also applies to exclusion of patients with positive antiphospholipid antibodies in whom the presence of cryoglobulins are more likely. Other probable explanation includes the use of immunosuppressive therapy in most of our studied patients which may have its impact on the occurrence of seronegativity. Lastly, cryoglobulins may be falsely negative in some patients due to the strict precautions needed for sample collection and analysis.

CONCLUSION

Cryoglobulinemia is not related to pathological markers of disease activity in the renal biopsies of patients with proliferative LN.

Conflict of Interest: Authors declare no conflicts of interest.

REFERENCES:

1. **Dooley MA, Aranow C, Ginzler EM.** Review of ACR renal criteria in systemic lupus erythematosus. Lupus 2004; 13:857-60.

2. **Kasitanon N, Magder LS, Petri M.** Predictors of survival in systemic lupus erythematosus. Medicine 2006; 85:147-56.

3. **Ward MM, Pyun E, Studenski S.** Mortality risks associated with specific clinical manifestations of systemic lupus erythematosus. Arch Intern Med 1996; 156:1337-44.

4. **Alarcon GS, McGwin G Jr, Petri M, et al.** Baseline characteristics of a multiethnic lupus cohort: PROFILE. Lupus 2002; 11:95-101.

5. **Gutiérrez S, Petiti JP, De Paul AL, et al.** Lupusrelated podocytopathy: Could it be a new entity within the spectrum of lupus nephritis. Nefrologia 2012; 32:245-6.

6. **Najafi CC, Korbet SM, Lewis EJ, et al.** Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. Kidney Int 2001; 59:2156-63.

7. **Markowitz GS, D'Agati VD.** The ISN/RPS 2003 classification of lupus nephritis: An assessment at 3 years. Kidney Int 2007; 71:491-5.

8. **Dammacco F, Sansonno D, Piccoli C, et al.** The cryoglobulins: an overview. Eur J Clin Invest 2001; 31:628-38.

9. **Harel S, Mohr M, Jahn I, et al.** Clinico-biological characteristics and treatment of type I monoclonal cryoglobulinaemia: a study of 64 cases. Br J Haematol 2015; 168:671-8.

10. **Terrier B, Karras A, Kahn JE, et al.** The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. Medicine 2013; 92:61-8.

11. **Saadoun D, Sellam J, Ghillani-Dalbin P, et al.** Increased risks of lymphoma and death among patients with non-hepatitis C virus-related mixed cryoglobulinemia. Arch Intern Med 2006; 166:2101-8.

12. **Trejo O, Ramos-Casals M, García-Carrasco M, et al.** Cryoglobulinemia: study of etiologic factors and clinical and immunologic features in 443 patients from a single center. Medicine 2001; 80:252-62.

13. **Adu D, Williams DG.** Complement activating cryoglobulins in the nephritis of systemic lupus erythematosus. Clin Exp Immunol 1984; 55:495-501.

14. **Roberts JL, Wyatt RJ, Schwartz MM, et al.** Differential characteristics of immune-bound antibodies in diffuse proliferative and membranous forms of lupus glomerulonephritis. Clin Immunol Immunopathol 1983; 29:223-41.

15. **García-Carrasco M, Ramos-Casals M, Cervera R, et al.** Cryoglobulinemia in systemic lupus erythematosus: prevalence and clinical characteristics in a series of 122 patients. Semin Arthritis Rheum 2001; 30(5):366-73.

16. **Barrera-Vargas A, Antiga-López FJ, Rangel-Patiño J, et al.** AB0546 Cryoglobulinemia in systemic lupus erythematosus: clinical and immunological features. Ann Rheum Dis 2018; 77(2):1429.