

Serum cryoglobulins and disease activity in systematic lupus erythematosus

Mansoor Karimifar, Samaneh Pourajam¹, Afshin Tahmasebi¹, Peyman Mottaghi

Departments of Rheumatology, School of Medicine, ¹Internal Medicine, School of Medicine and Student Research Committee, Isfahan University of Medical Sciences, Isfahan, Iran

Background: To determine the prevalence of cryoglobulins in Iranian patients with systemic lupus erythematosus (SLE) and evaluate the correlation of cryoglobulins with disease activity in these patients. **Materials and Methods:** In a cross-sectional study, we investigated 80 consecutive women who fulfilled the 1982 revised criteria of the American College of Rheumatology for the classification of SLE. All the patients had undergone a medical interview and general physical examination by a rheumatologist for clinical and serologic characteristics of SLE. For the determination of cryoglobulins, sera were collected by a standard protocol at 37°C, and after incubation at 4°C for seven days, the level of cryoglobulins was estimated for each patient. **Results:** Cryoglobulins were detected in the sera of 39 (48.8%) patients. All of these patients had cryocrit over 5%. Disease was active in 30 patients [SLEDAI ≥6 (DAI: disease activity index)] and inactive in 50 (SLEDAI <6). There was no significant difference between active and inactive patients for the presence of serum cryoglobulins ($r = 0.086$, $P = 0.56$). A significant positive correlation was observed between antinuclear antibody (ANA), anti-dsDNA (dsDNA: Double-stranded deoxyribonucleic acid), CH50 (CH50: total hemolytic complement assay), and C-reactive protein (CRP) ($r = 0.21$, $P = 0.004$, $r = 0.65$, $P = 0.001$, $r = 0.45$, $P = 0.023$, $r = 0.38$, $P = 0.036$, respectively). Hepatitis C virus (HCV) infection was not detected in any of the SLE patients. **Conclusion:** Although the presence of cryoglobulins in the SLE patients correlated with positive anti-ds DNA and low CH50, it could not be predict activity of the disease.

Key words: Cryoglobulinemia, disease activity index, systemic lupus erythematosus

INTRODUCTION

Although the term 'lupus erythematosus' was introduced by 19th century physicians to describe the skin lesions, it took almost 100 years to realize that the disease is systemic and spares no organ and that it is caused by an aberrant autoimmune response.^[1]

The diversity of clinical features includes articular and mucocutaneous, renal, hematologic, and central nervous system abnormalities.^[2] However, it is thought that this condition can be divided into more homogeneous subsets of pathogenic, therapeutic, and prognostic significance.^[3-6]

Cryoglobulinemia is a rare disease that develops as a result of the presence of high levels of abnormal proteins, called cryoglobulins, in the blood.^[7,8] In cold temperatures, these proteins clump together and block blood vessels, potentially causing a wide range of complications. There are several types of cryoglobulinemia that are classified based on the type of the abnormal protein that is present in the blood. The three types of cryoglobulinemia known to exist are referred to as type I, type II, and type III. The main difference between these types is the nature of the

cryoglobulin antibodies and the factors that caused them to develop. In most cases, type I is associated with cancers of the immune system or the blood. Type II and type III usually develop in people who have a chronic inflammatory condition such as systemic lupus erythematosus (SLE) or hepatitis C.^[7,8]

Cryoglobulinemia has many potential symptoms, because the presence of cryoglobulins in the blood can affect almost any organ or tissue type. This is because the precipitation of the proteins can block any of the blood vessels of the body; so, the symptoms of the disease depend on the site or sites of blood vessel blockage. Even so, certain effects are more common than others. The common symptoms of cryoglobulinemia include fatigue, muscle pain, joint pain, difficulty in breathing, skin ulceration, and death of large patches of skin cells. These symptoms are quite general and can develop regardless of the specific organ or organs involved. Kidney disease and liver disease are relatively common consequences. These organs are the most likely to be affected by levels of blood cryoglobulins because of their roles in filtering the blood of waste products.^[9,10] So far, the cryoglobulins and their association with lupus activity in patients in Isfahan had not been investigated; hence, the present study was necessary.

Address for correspondence: Dr. Mansoor Karimifar, Rheumatology Dept., School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: mansoor_karimifar@yahoo.com

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The aim of our present study was to determine the prevalence and nature of cryoglobulins in a number of SLE patients and to evaluate the association between the cryoglobulins and disease activity in these patients.

MATERIALS AND METHODS

This was a cross-sectional study in Isfahan, Iran and was conducted from September 2010 to May 2011. We studied 80 women with SLE [mean \pm standard deviation (SD) age: 33.3 ± 9.6 years; 15 to 60 years] in our unit. All the patients fulfilled the 1982 revised criteria of the American College of Rheumatology for the classification of SLE.^[11] All had documented medical histories and underwent a medical interview and a routine general physical examination by a rheumatologist. Clinical and serologic characteristics of all these patients were collected on a protocol form. Clinical disease activity was assessed according to the SLE disease activity index (SLEDAI) score.^[12]

Blood samples were obtained and kept at 37°C for 30 minutes before separation. Serum was prepared by centrifuging for 10 minutes at 2,500 rpm.^[7,8] Fresh centrifuged serum was incubated at 4°C for seven days after collection and examined for cryoprecipitate. The cryocrit was obtained by centrifuging at 2,000 rpm (750 g) for 30 minutes at 4°C. The cryoprecipitate was diluted in warm saline for one hour. Finally, dissolved cryoprecipitate was identified by agarose gel electrophoresis and immunofixation.^[7,8] Other immunologic tests included antinuclear antibodies (ANA) [enzyme-linked immunosorbent assay (ELISA)], antibodies to double-stranded DNA (anti-dsDNA) (ELISA), C-reactive protein (CRP) (latex fixation), and erythrocyte sedimentation rate (ESR). Complement factors (C3 and C4) were estimated by nephelometry (Behring BNA nephelometer) and total hemolytic complement assay (CH50) by Lachmann's hemolytic technique. Hepatitis C virus (HCV) antibodies were determined by ELISA.

Statistical analysis

We used conventional Chi-square and Fisher's exact tests to analyze qualitative differences. For the calculation of correlation coefficients, Pearson's correlations were used. For comparison of quantitative parameters, Student's *t*-test was used in large samples of similar variance, and the nonparametric Mann-Whitney U test for small samples. A value of $P < 0.05$ indicated statistical significance. The odds ratio (OR) was calculated to assess the risk of appearance of each variable, with a confidence interval (CI) of 95%. This statistical analysis was performed by the SPSS program (SPSS Inc, Chicago, Illinois) with the information stored in the database program.

RESULTS

Age of the patients was between 15 and 60 years (mean \pm SD: 33.3 ± 9.6 years) and the mean duration of disease since diagnosis was 66 months (1 to 240 months). Cryoglobulins were detected in the sera of 39 women with SLE (48.8%) with a minimum value of 1.4 $\mu\text{g/mL}$ to a maximum value of 32.5 $\mu\text{g/mL}$. To assess levels of cryoglobulins NovaTeinBio kit (USA) was used and cryoglobulinemia was defined as serum cryoglobulin levels $>20 \mu\text{g/mL}$ in this kit).

Figure 1 shows the correlation between the SLEDAI score and cryoglobulins. There was no significant correlation between cryoglobulins and SLEDAI ($r = 0.043$, $P = 0.17$). Correlation was also tested between the cryoglobulins and other serological markers. Again, no significant correlation could be demonstrated between cryoglobulins and C3 ($r = 0.108$, $P = 0.32$), C4 ($r = 0.176$, $P = 0.38$), and ESR ($r = 0.298$, $P = 0.65$). However, a significant positive correlation was observed between cryoglobulins and anti-dsDNA ($r = 0.65$, $P = 0.001$), ANA ($r = 0.21$, $P = 0.004$), CH50 ($r = 0.45$, $P = 0.023$), and CRP ($r = 0.38$, $P = 0.036$). On the basis of the SLEDAI, 30/80 (37.5%) patients were identified with lupus activity (SLEDAI ≥ 6). Median cryoglobulin was 27.8 $\mu\text{g/mL}$ in active disease and 25.6 $\mu\text{g/mL}$ in nonactive disease [Figure 2]. There was no significant difference in serum cryoglobulins between active and nonactive patients ($r = 0.086$, $P = 0.56$).

There was no significant difference in the frequency of several clinical manifestations between SLE patients with cryoglobulinemia and those without it [Table 1], but a significant positive correlation was observed between other markers such as ANA, anti-dsDNA, and CH50 [Table 1]. On the other hand, a decrease in C3 and C4 and an increased percentage of clinical features (renal involvement, arthritis, and hematologic manifestation)

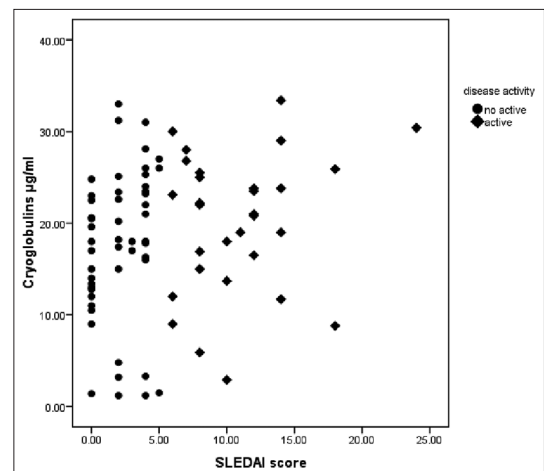


Figure 1: Distribution and correlation between systemic lupus erythematosus disease activity index (SLEDAI) score and cryoglobulins

appeared to be more common in the cryoglobulinemic group, although the difference did not reach statistical significance [Table 1].

All of the 39 SLE patients showed a high percentages of cryocrit (>5%) and for these patients, cryoprecipitates were separated by high-resolution agarose electrophoresis. Eighteen cryoprecipitates were type II mixed cryoglobulins containing monoclonal IgMκ with polyclonal IgG (IgG: immunoglobulin G), four cryoprecipitates were a mixture of IgG and IgA, and two precipitates showed all the three IgG, IgA, and IgM immunoglobulins. The electrophoresis

of the remaining cryoprecipitates identified a monoclonal IgGλ.

HCV infection was not detected in any of the SLE patients.

DISCUSSION

The role of cryoglobulins in the pathogenesis of rheumatic diseases is being widely investigated; indeed, SLE is now being regarded as an autoimmune disease by an increasingly large number of markers. The study of the seropathology and the presence and nature of the cryoglobulins in SLE was, therefore, undertaken in the present study.

A cryoglobulin is a serum protein or proteins that precipitate/s when serum is incubated at a temperature of less than 37°C. Cryoglobulins undergo reversible precipitation at cold temperatures. Although fibrinogen may cryoprecipitate, this report describes only immunoglobulins. The cold-induced precipitation of serum proteins was first described in 1933,^[13] and Lerner and Watson introduced the term cryoglobulinemia in 1947.^[14] In 1966, Meltzer and Franklin^[15] described the typical clinical symptoms associated with cryoglobulinemia, particularly the triad of purpura, arthralgia, and weakness. The existence of circulating cryoglobulins (cryoglobulinemia) is not always related to the presence of symptomatology, and we use the term cryoglobulinemic syndrome when patients with cryoglobulinemia have clinical manifestations. Since the initial report in 1990 of the association between mixed cryoglobulinemia (MC) and HCV infection, it has become clear that most of the so-called 'essential' cryoglobulinemias are in fact associated with HCV infection. The discovery of the relationship between HCV infection and MC shows the striking association between a viral infection and an autoimmune disease and, moreover, a potential link between autoimmune and lymphoproliferative disorders.

Cryoglobulinemia has been studied in some systemic autoimmune diseases, such as rheumatoid arthritis,^[16,17] systemic sclerosis,^[18] polyarteritis nodosa,^[19] and, especially, in Sjögren's syndrome (SS).^[20] The clinical significance of cryoglobulinemia in SLE has been studied little, and its prevalence has ranged from 16 to 83% in a small series of patients.^[21-27] The cryoproteins found in SLE contain immunoglobulins, mostly IgG and IgM, and complement components.^[28-31] Many studies have been performed with the aim of defining the antibody specificity and the presumptive antigens in the cryoglobulins. The most frequently found autoantibodies in cryoprecipitates of patients with SLE were anti-dsDNA, anti-single-stranded anti-DNA and, rarely, antiribonucleoprotein. These autoantibodies are more concentrated in cryoprecipitates than in serum and are correlated with the autoantibodies

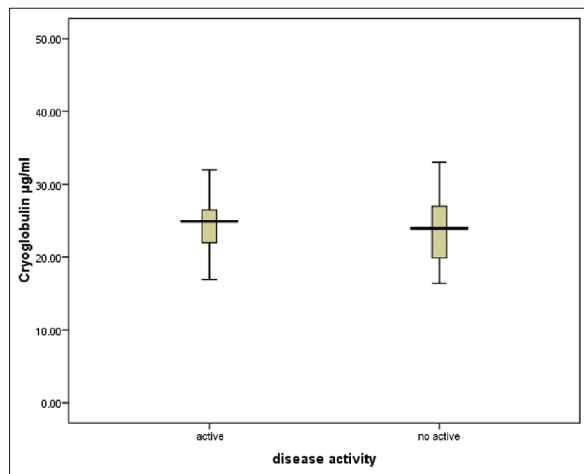


Figure 2: Median serum cryoglobulins according to systemic lupus erythematosus disease activity index (SLEDAI)

Table 1: Clinical and immunological features of systemic lupus erythematosus in patients

	SLE patients with cryoglobulinemia (n=39) (%)	SLE patients without cryoglobulinemia (n=41) (%)	P value
Mucocutaneous manifestations	13 (33.4)	13 (31.2)	NS
Hematological manifestations	5 (12.9)	5 (12.2)	NS
Renal involvement	17 (43.6)	13 (31.2)	NS
Arthritis	22 (56.5)	2 (4.9)	NS
Pericardial manifestations	0 (0)	1 (2.5)	NS
Anti-dsDNA	22 (56.5)	2 (4.9)	0.001
ANA	28 (71.8)	19 (46.4)	0.004
C3	18 (46.2)	15 (36.6)	NS
C4	22 (56.5)	17 (41.5)	NS
CH50	32 (81.1)	25 (60.9)	0.023
Elevation of ESR	8 (20.6)	10 (24.4)	NS
CRP	2 (30.1)	6 (14.5)	0.036
Active patients (SLEDAI≥6)	17 (43.6)	13 (31.8)	NS

NS=Not significant; Anti-ds DNA=Anti-double-stranded DNA; ANA=Antinuclear antibodies; C3=Complement 3; C4=Complement 4; CH50=Total hemolytic complement assay; ESR= Erythrocyte sedimentation rate; CRP= C-reactive protein; SLE=Systemic lupus erythematosus; SLEDAI=SLE disease activity index

found in the elution of glomeruli of patients with lupus nephritis.^[21] In addition, we found a type I cryoglobulinemia in 18 of our SLE patients. Usually, patients with SLE show type II or type III cryoglobulinemia,^[26,29] although type I cryoglobulinemia has been described infrequently.^[29]

In this study, we found a prevalence of 48.8% of cryoglobulins in a large number of SLE patients, all of whom showed high amounts of circulating cryoglobulins (cryocrit >5%). Gripenberg *et al.*^[23] found low levels of cryoglobulins (<0.05 g/L) in 81% of the SLE patients analyzed. Additionally, some studies have found an association between the amount of cryoprecipitates and the disease activity in SLE.^[24,28] We also analyzed the clinical SLE features according to the presence or absence of cryoglobulinemia and found a higher prevalence of arthritis in cryoglobulinemic SLE patients but this difference did not reach statistical significance. In previous studies, cryoglobulinemia has been related to disease activity and severity (particularly nephritis),^[21,22,25] although other researchers found no significant differences.^[23] We found no significant increase of renal involvement in our SLE cryoglobulinemic patients. The presence of cryoglobulinemia appeared to have no influence on the renal involvement of our SLE patients.

Furthermore, we found a higher frequency of some immunologic markers in cryoglobulinemic SLE patients [CRP, anti-dsDNA, ANA, and CH50 (hypocomplementemia)]. The association between hypocomplementemia and cryoglobulins is well known. Adu and Williams^[26] showed the ability of SLE cryoglobulins to activate complements *in vitro*, suggesting that these immune complexes can activate complements *in vivo* and thus may contribute to tissue damage in this disease. Roberts *et al.*^[27] suggested that in SLE patients with diffuse proliferative glomerulonephritis, immune-bound antibodies in cryoglobulins and glomerular-immune deposits appear to activate complements via the classic and alternative pathways. Interestingly, we found a higher frequency of CRP in cryoglobulinemic SLE patients, probably related to the CRP activity of some cryoglobulinemic components. CRP might be an immunologic marker that suggests the existence of cryoglobulinemia in SLE patients.

Finally, we analyzed the possible role of HCV in the cryoglobulinemia of our SLE patients. The last study of this subject was performed before the development of a test for the detection of anti-HCV antibodies in 1989. Given the strong association between MC and HCV infection,^[32-35] the cryoglobulinemia observed in some SLE patients might be associated with HCV infection. In this study, not one of the SLE patients with or without cryoglobulinemia had HCV infection.

To summarize, we found that anti-dsDNA, ANA, CRP, and hypocomplementemia correlated with the presence of cryoglobulins in the SLE patients. These finding may identify a subset of SLE patients with cryoglobulinemia. The present study may thus help in the understanding of immunochemical parameters of SLE which are involved in systemic immunoinflammation.

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