THE ASSOCIATION BETWEEN ANTI-U1-RNP ANTIBODY AND SKIN LESIONS, MUSCLE INVOLVEMENT AND PERIPHERAL VASCULAR INJURY IN PATIENTS WITH SYSTEMIC SCLEROSIS

Vu Xuan Huong[1], Le Huu Doanh[2,3], Hoang Thi Phuong[3], Do Thi Thu Hien[3], and Vu Huy Luong[2,3,*]

ABSTRACT

Objectives: To evaluate the relationship between anti-U1-RNP antibodies and skin, muscle, peripheral vascular, and renal damage in patients with systemic sclerosis.

Subjects and methods: A cross-sectional descriptive study was conducted on 55 patients diagnosed with systemic sclerosis according to the criteria of the American College of Rheumatology (ACR) and the European League Against Rheumatism (2013) at the National hospital of Dermatology and Venereology from September 2021 to September 2022. Patients were tested for anti-U1-RNP antibodies in their blood using the ANA 23 profile test.

Results: The positivity rate for anti-U1-RNP antibodies was 14.5%. Among skin lesions, the most common manifestation was sclerodactyly (87.3%), followed by poikiloderma (47.3%), and less frequently, digital edema (27.3%). The average modified Rodnan skin score in the systemic sclerosis group was 21.2 ± 6.8, with the anti-U1-RNP positive group scoring higher (27.3 ± 6.1) than the negative group (20.2 ± 5.8) (p = 0.002). The incidence of peripheral vascular damage, including the rate of periungual telangiectasia and periungual hemorrhage, was higher in the anti-U1-RNP positive group compared to the negative group (p = 0.018 and p = 0.034, respectively). The rate of elevated muscle enzymes (CK > 190 U/l) in the anti-U1-RNP positive group (75.0%) was also higher than in the negative group (17.4%) (p = 0.002). The incidence of elevated serum creatinine and proteinuria in the systemic sclerosis group with positive anti-U1-RNP antibodies was higher than in the negative group (p = 0.018 and p = 0.029, respectively).

Conclusions: Patients with systemic sclerosis who test positive for anti-U1-RNP antibodies tend to have higher rates of skin lesions, peripheral vascular damage, muscle damage, and renal damage compared to those who test negative.

[1]Hanoi Dermatology Hospital
[2]Hanoi Medical University
[3]National hospital of Dermatology and Vereneology
**Correspondence: Email: huyluong84@gmail.com*
Received 12 May 2023
Revised 11 July 2023
Accepted 28 August 2023
DOI: 10.56320/tcdlhvn.46.196

Keywords: *ANA 23 profile, anti-U1-RNP antibodies, organ damage, systemic sclerosis.*

1. INTRODUCTION

Systemic Sclerosis (SSc) is a common autoimmune disease, ranking second after

systemic lupus erythematosus, typically occurring in individuals aged 30 to 50 and affecting women more than men. The incidence of this disease is on the rise within the group of connective tissue diseases. Each year, over 400 patients visit the National Hospital of Dermatology and Venereology for examination and treatment of systemic sclerosis[1]. The etiology and pathogenesis of the disease remain unclear, and treatment poses many challenges. Clinical manifestations are diverse, with skin lesions accompanied by damage to multiple internal organs, which can be a primary cause of severe disease and mortality.

The autoantibody profile in systemic sclerosis is also quite extensive. Some autoantibodies not only serve as markers for diagnosis but also play a role in predicting organ damage.[2] Among these, anti-U1-RNP antibodies have been well studied regarding their structure and their presence in various autoimmune connective tissue diseases. Several global studies have shown that anti-U1-RNP antibodies are associated not only with skin, muscle, and peripheral vascular damage but also with damage to internal organs and survival prognosis. A study by Sobanski et al. (2015) involving 342 patients with autoimmune connective tissue diseases and pulmonary hypertension, focusing on systemic sclerosis patients, indicated that anti-U1-RNP antibodies were related to skin lesions, lung damage, and patient survival, while hemodynamic parameters showed no significant differences[3]. Additionally, research by Wanlong Wu et al. (2018) on 8,391 systemic sclerosis patients, including 408 who were positive for anti-U1-RNP antibodies, found that the positive group had higher rates of synovial membrane inflammation, pulmonary artery hypertension, interstitial lung damage, proteinuria, elevated sedimentation rate, and anemia compared to the negative group[4]. However, in Vietnam, data on anti-U1-RNP antibodies in autoimmune connective tissue diseases in general and systemic sclerosis in particular are still limited. According to author Nguyễn Thị Thảo Nhi (2019), the prevalence of anti-U1-RNP antibodies in systemic sclerosis patients is 17.5%, primarily at moderate levels. The mean pulmonary artery pressure in the anti-U1-RNP positive group was significantly higher than in the negative group[5]. This study aims to further evaluate the relationship between anti-U1-RNP antibodies and damage to specific organs in systemic sclerosis.

2. SUBJECTS AND METHODS

2.1. Study subjects

Inclusion criteria: Patients diagnosed with systemic sclerosis according to the criteria of the American College of Rheumatology (ACR) and the European League Against Rheumatism (2013)[6] who visited the National hospital of Dermatology and Venereology for examination and treatment from September 2021 to September 2022; patients must agree to participate in the study and undergo testing for anti-U1-RNP antibodies using the ANA 23 profile test along with other supporting tests. Exclusion Criteria: Patients who do not meet the diagnostic criteria for systemic sclerosis as defined by the American College of Rheumatology (ACR) and the European League Against Rheumatism (2013), those who do not agree to participate in the study, or those who have already been included in the study during a previous visit.

2.2. Study methods

Study design

This is a cross-sectional descriptive study conducted from September 2021 to September 2022 at the National hospital of dermatology and venereology. Convenience sampling was used until the end of the study period, with a minimum of 30 patients.

Procedures

Patients who visit the National Hospital of Dermatology and Venereology and are diagnosed with systemic sclerosis will have their medical records created using a standard form. Following this, patients will undergo testing for anti-U1-RNP antibodies using the ANA 23 profile test, along with other examinations to assess organ damage, including capillary nailfold dermoscopy, serum CK levels, blood creatinine levels, and a complete urinalysis.

In this study, anti-U1-RNP antibodies were detected using the ANA 23 profile kit from EUROIMMUNE (Lübeck, Germany), employing an immunoblot technique based on the antigen-antibody binding mechanism. The membrane is coated with pure antigens arranged in parallel lines, which combine with specific antibodies in the patient's blood. These are then colored and displayed as colored bands on the strip.

The results of the ANA 23 profile tests are presented in both qualitative and semi-quantitative formats: Qualitative: Positive or negative. Semi-quantitative: Signal density ranging from 0 to 100 is categorized into three positivity levels as follows:

0 - 10: Negative

11 - 25: Positive 1+

26 - 50: Positive 2+

50: Positive 3+

Patients are assessed for skin lesions (digital edema, sclerodactyly, poikiloderma, calcinosis) and skin thickness. Skin thickness is evaluated using a modified Rodnan skin score across 17 areas of skin through a pinch technique (using either two thumbs or one thumb and one index finger to pinch). The degree of skin thickening at each site is categorized into three levels, scoring from 0 to 3, with a maximum total score of 51 points:

0 points for no skin thickening.

1 point for mild thickening.

2 points for moderate thickening.

3 points for severe thickening (skin thickened to the bone, with almost no mobility).

The Rodnan score assessment is highly subjective. To reduce potential errors, we provided training on how to evaluate the Rodnan score for the doctors involved in the assessments, who also performed direct evaluations on each patient.

Data were compiled to calculate rates and determine the relationship between anti-U1-RNP antibodies and damage to certain organs in systemic sclerosis.

Statistical analysis

The collected data were entered, managed, and processed using SPSS software version 20.0. Appropriate statistical tests were employed, including the McNemar chi-square test for qualitative variables within the same group and the chi-square test for two qualitative variables from different groups. If the theoretical expectation was less than 5, Fisher's exact test was used. For quantitative variables, a t-test was applied to compare two independent samples. Statistical significance was defined as $p < 0.05$.

2.3. Ethics

Patients were provided with clear information and agreed to participate in the study. All personal information of patients will be kept confidential. Patients who decline to participate will still receive thorough examination, consultation, and treatment. All collected data will be used solely for research purposes and will not be utilized for any other purpose. This study was approved by the Ethics Committee and received consent from the hospital management and department heads at the National Hospital of Dermatology and Venereology, as stated in decision number 367/ HĐĐĐ-BVDLTW.

3. RESULTS

3.1. Common skin lesions in patients with systemic sclerosis

Among the 55 patients participating in the study, there were 39 female patients (accounting for 70.9%) and 16 male patients (accounting for 29.1%). The average age of onset was 49.8 ± 12.3 years, with the youngest being 12 years old and the oldest 73 years old. The percentage of patients testing positive for anti-U1-RNP antibodies was 14.5%, primarily with moderate positivity at level 2+ (37.5%) and strong positivity at level 3+ (37.5%).

Table 1. Common Skin Lesions in Patients with Systemic Sclerosis (SSc)

No.	Manifestations	Anti-U1-RNP antibodies						p
		Positive (n = 8)		Negative (n = 47)		Total (N = 55)		
		n	%	n	%	n	%	
1	Finger edema	3	37.5	12	25.5	15	27.3	0.669*
2	Finger sclerosis	6	75.0	42	89.4	48	87.3	0.267*
3	Poikiloderma	6	75.0	20	42.6	26	47.3	0.131*
4	Fingertip ulcers	2	25.0	9	19.1	11	20.0	0.654*
5	Calcinosis	0	0.0	0	0.0	0	0.0	

**: Fisher's exact test.*

In patients with systemic sclerosis, finger sclerosis was the most common manifestation (87.3%), followed by poikiloderma (47.3%), with finger edema (27.3%) and fingertip ulcers (20.0%) being less frequent. No patients in the study presented with skin calcinosis. There was no significant difference in the prevalence of these symptoms between the U1-RNP antibody-positive and antibody-negative groups.did not differ between the groups positive and negative for anti-U1-RNP antibodies.

3.2. Comparison of the mean mRodnan score in systemic sclerosis patients with positive and negative anti-U1-RNP antibodies

Table 2. Comparison of the mean mRodnan score in systemic sclerosis patients with positive and negative anti-U1-RNP antibodies

No.	Anti-U1-RNP antibodies	mRodnan score (X ± SD)	p
1	Positive (n = 8)	27.3 ± 6.1	0.002†
2	Negative (n = 47)	20.2 ± 5.8	
3	Total (N = 55)	21.2 ± 6.8	

†: Independent t-test.

The mRodnan score in our study's systemic sclerosis patients was 21.2 ± 6.8. Among them, the mRodnan score in the group of systemic sclerosis patients positive for anti-U1-RNP antibodies (27.3 ± 6.1) was higher than in the group negative for anti-U1-RNP antibodies (20.2 ± 5.8), with a statistically significant difference (p = 0.002).

3.3. Association between anti-U1-RNP antibodies and peripheral vascular damage

Table 3. Association between anti-U1-RNP antibodies and peripheral vascular damage

Characteristic	Anti-U1-RNP antibodies						p
	Positive (n = 8)		Negative (n = 47)		Total (N = 55)		
	n	%	n	%	n	%	
Raynaud's phenomenon	4	50.0	17	36.2	21	38.2	0.464*
Nailfold telangiectasia	7	87.5	18	38.3	25	45.5	**0.018***
Nailfold hemorrhage	3	37.5	3	6.4	4	8.3	**0.034***

**: Fisher's exact test.*

The prevalence of nailfold telangiectasia and nailfold hemorrhage was higher in the anti-U1-RNP antibody-positive group compared to the negative group, with statistically significant differences (p = 0.018 and p = 0.034, respectively).

3.4. Rate of elevated muscle enzymes in patients with positive and negative anti-U1-RNP antibodies

Table 4. Rate of elevated muscle enzymes in patients with positive and negative anti-U1-RNP antibodies

Anti-U1-RNP antibodies	Elevated muscle enzymes (CK > 190 U/l)		p
	n	%	
Positive (n = 8)	6	75.0	0.002*
Negative (n = 47)	8	17.4	
Total (N = 55)	14	25.9	

**: Fisher's exact test.*

The prevalence of elevated muscle enzyme levels (CK > 190 U/l) was higher in the group positive for anti-U1-RNP antibodies compared to the negative group, with a statistically significant difference of p = 0.002.

3.5. Relationship between anti-U1-RNP antibodies and renal damage

Table 5. Relationship between anti-U1-RNP antibodies and renal damage

Renal Damage Index	Anti-U1-RNP antibodies						p
	Positive (n = 8)		Negative (n = 47)		Total (N = 55)		
	n	%	n	%	n	%	
Increased Blood Creatinine	3	37.5	2	4.3	5	9.1	**0.018***
Proteinuria	4	50.0	6	12.8	10	18.2	**0.029***

**: Fisher's exact test.*

The rates of increased blood creatinine and proteinuria are higher in patients with systemic sclerosis who are positive for anti-U1-RNP antibodies compared to those who are negative, with statistically significant differences (p = 0.018 and p = 0.029, respectively).

4. DISCUSSION

The skin manifestations of systemic sclerosis patients are quite varied. However, our study focused on evaluating commonly encountered and easily detectable skin manifestations. According to Table 3.1, among the common skin manifestations (finger edema, sclerodactyly, poikiloderma, fingertip ulcers, and calcinosis), sclerodactyly is the most frequent manifestation, and no symptom was related to the presence of anti-U1-RNP antibodies. The rates of finger edema, poikiloderma, and fingertip ulcers were higher in patients with positive anti-U1-RNP antibodies compared to those with negative antibodies, but this difference was not statistically significant. Our findings differ from those of Thân Trọng Tuỳ (2014), where finger edema was the most common symptom at 63.8%[8]. This discrepancy may be due to differences in sampling methods and the fact that Tuỳ's study included mostly outpatients with early, mild skin damage, whereas our study included more inpatients with severe, less elastic skin damage and pigmentary disorders.

To assess skin damage in systemic sclerosis patients, we used the modified Rodnan skin score (mRodnan). The results showed that the mRodnan score in patients with positive anti-U1-RNP antibodies (27.3 ± 6.1) was higher than in those with negative antibodies (20.2 ± 5.8), with a statistically significant difference (p = 0.002) (Table 3.2). Other studies on skin thickness in systemic sclerosis patients with limited and diffuse skin damage reported mRodnan scores of 14.3 and 36.2 (Phạm Thị Tuyến 2012); 9.1 and 22.8 (Kevin Keen 2012); 3.1 and 13.2 (Thân Trọng Tùy 2014); 13.7 ± 8.8 (Nguyễn Thị Hoa 2015); 13.6 ± 9.4 (Nguyễn Thị Thảo Nhi 2019) [1,7-10]. Our mRodnan score is higher compared to some other studies. The variability between studies could be due to different sampling methods and the subjective nature of mRodnan scoring.

Peripheral vascular damage is very common in systemic sclerosis, presenting in various degrees from simple Raynaud's phenomenon

to severe complications like atrophic scars, ulcers, and digital necrosis. Our study assessed peripheral vascular damage through nailfold capillary changes and Raynaud's phenomenon. These manifestations often appear early, persist, and change with disease progression, and can be easily detected through clinical examination or dermoscopy, responding well to early treatment. Results in Table 3.3 show that Raynaud's phenomenon was more common in patients with positive anti-U1-RNP antibodies compared to those with negative antibodies (50.0% vs. 36.2%), but this difference was not statistically significant. However, there was a significant association between anti-U1-RNP antibodies and nailfold capillary dilation and hemorrhage, with rates higher in the positive group (87.5% and 37.5%, respectively) compared to the negative group (38.3% and 6.4%, respectively). Abnormal nailfold capillary findings are relatively common in autoimmune connective tissue diseases and can be detected through clinical examination or dermoscopy. Therefore, evaluating nailfold capillaries should be a routine practice when examining autoimmune patients in general and systemic sclerosis patients in particular to avoid missing diagnoses and to ensure early treatment.

Muscle damage manifestations were evaluated through physical examination and patient interviews about symptoms including muscle pain at rest, muscle pain on palpation, muscle weakness, and muscle atrophy. Elevated muscle enzyme levels were also used to assess muscle damage. According to Table 3.4, the rate of elevated muscle enzymes (CK > 190 U/l) was higher in patients with positive anti-U1-RNP antibodies compared to those with negative antibodies, with a statistically significant difference. There are few studies on the relationship between anti-U1-RNP and muscle damage worldwide. Virginia D Steen's study (2005)[11] on patients with systemic sclerosis from the Pittsburgh scleroderma database (1980 - 1995) found that myositis was quite common in scleroderma patients (27%) and second only to those with anti-PM-Scl antibodies (58%).[12] Our results also show an association between anti-U1-RNP antibodies and muscle damage, particularly on muscle enzyme tests in systemic sclerosis patients. However, further studies with larger and longer sample sizes are needed to strengthen these findings.

Renal damage often progresses silently and is sometimes detected through renal crises or early changes in laboratory tests. Our study assessed renal damage through blood creatinine levels and proteinuria. Results in Table 3.5 show that 5 patients with systemic sclerosis had elevated blood creatinine (9.1%) and 10 had proteinuria (18.2%). The rates of elevated blood creatinine and proteinuria were higher in patients with positive anti-U1-RNP antibodies compared to those with negative antibodies, with statistically significant differences ($p = 0.018$ and $p = 0.029$, respectively). This result is consistent with Wanlong Wu's (2018) study, which found higher proteinuria rates in the positive group (12.6% vs. 5.6%).[4] Renal damage is difficult to detect clinically at an early stage, but changes in laboratory tests can help physicians assess early renal damage in autoimmune patients. Therefore, in addition to clinical examination, urine and blood tests are necessary for patients.

Our study is a cross-sectional description, so it only assesses organ damage characteristics at one point in time and does not evaluate changes over time. The 23 ANA Profile test is qualitative, so its accuracy has some limitations. However, to minimize errors, we used standard tests approved

by the Ministry of Health, and researchers directly conducted examinations and data collection. The preliminary results indicate that anti-U1-RNP antibodies are associated with skin, muscle, peripheral vascular, and renal damage in systemic sclerosis patients.

5. CONCLUSION

Patients with systemic sclerosis who are positive for anti-U1-RNP antibodies tend to have higher rates of skin damage, peripheral vascular damage, muscle damage, and renal damage compared to those who are negative.

REFERENCES

1. Phạm Thị Tuyến. Nghiên cứu đặc điểm lâm sàng và mối liên quan giữa tổn thương da với nội tạng ở bệnh nhân xơ cứng bì hệ thống. Luận văn Bác *sĩ* chuyên khoa cấp II, Trường Đại học Y Hà Nội, Hà Nội. 2012:4-5.

2. Mulhearn B, Tansley SL, McHugh NJ. Autoantibodies in connective tissue disease. Best Pract Res Clin Rheumatol. 2020;34(1):101462. doi:10.1016/j.berh.2019.101462.

3. Sobanski V, Giovannelli J, Lynch BM, et al. Characteristics and Survival of Anti-U1 RNP Antibody-Positive Patients With Connective Tissue Disease-Associated Pulmonary Arterial Hypertension. Arthritis Rheumatol. 2016;68(2):484-493. doi:10.1002/art.39432.

4. Wu W, Hoederath P, Hachulla E, Airò P, Valentini G, Matucci Cerinic M, Cozzi F, Riemekasten G, Allanore Y, Carreira P, Jordan S, Distler O. Demographic and Clinical Features of Systemic Sclerosis Patients with Anti-U1RNP Antibodies: A European Scleroderma Trials and Research (EUSTAR) Analysis. Arthritis Rheumatol. 2018; 70 (suppl 9).

5. Koschik RW, Fertig N, Lucas MR, Domsic RT, Medsger TA. Anti-PM-Scl antibody in patients with systemic sclerosis. Clin Exp Rheumatol. 2012;30(2 Suppl 71):S12-16.

6. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. 2013;65(11):2737-2747. doi:10.1002/art.38098.

7. Keen KJ, van Eeden SF, Dunne JV. Limited Cutaneous and Diffuse Cutaneous Scleroderma: Circulating Biomarkers Differentiate Lung Involvement. Rheumatology. 2012;S1:006. doi: 10.4172/2161-1149.S1-006.

8. Thân Trọng Tuỳ. Đánh giá hiệu quả điều trị bệnh xơ cứng bì hệ thống bằng methotrexate. Luận văn Bác *sĩ* nội trú chuyên ngành da liễu, Trường Đại học Y Hà Nội, Hà Nội. 2014:40-41.

9. Nguyễn Thị Hoa. Mối liên quan giữa kháng thể kháng Topoisomerase I với tổn thương một số cơ quan trong bệnh xơ cứng bì hệ thống. Luận văn Bác *sĩ* nội trú chuyên ngành da liễu, Trường Đại học Y Hà Nội, Hà Nội. 2015:43-45.

10. Nguyễn Thị Thảo Nhi. Mối liên quan giữa đặc điểm lâm sàng và một số tự kháng thể trong bệnh xơ cứng bì hệ thống. Luận văn Thạc sĩ Y học chuyên ngành da liễu, Trường Đại học Y Hà Nội, Hà Nội. 2019:40-41.

11. Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum.2005;35(1):35-42. doi:10.1016/j.semarthrit.2005.03.005.

12. Pauling JD, Skeoch S, Paik JJ. The clinicoserological spectrum of inflammatory myopathy in the context of systemic sclerosis and systemic lupus erythematosus. Indian J Rheumatol. 2020;15(6):81-90. doi:10.4103/injr.injr_136_20.