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Original Article

Evaluation of Rare Antinuclear Antibody Patterns in a Tertiary Hospital in İzmir

Aslı Gamze Sener[1]

[1]Izmir Katip Celebi University Ataturk Training and Research Hospital, Medical Microbiology, Izmir, Turkey

Address for Correspondence: Asli Gamze Sener, **E-mail:** agsener@gmail.com
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Abstract

Objective: Antinuclear antibody (ANA) patterns are evaluated as nuclear, cytoplasmic or mitotic on HEp-2 cel l. Although some ANA patterns have been comprehensively studied such as homogenous, speckled or nucleolar staining patterns, rare antinuclear antibody patterns still require further assessment. In this study, the rare pattern was defined as 1% occurring ratio on indirect immunofluorescence assay. Rare ANA patterns were evaluated in a 6-year period retrospectively.

Material and methods: The study includes 41921 serum samples that different departments had sent to the tertiary Hospital's Laboratory of Medical Microbiology for ANA test between January 2010 and December 2015. Serum samples were studied in dilution of 1:100 using HEp-20-10/liver biochip (Monkey) and conjugated with specific antihuman IgG (Euroimmun AG, Lubeck, Germany). The fluorescence intensity was evaluated at x400 by immunofluorescence microscope (Eurostar III plus). Evaluation was performed as semi-quantitatively from 1+ to 4+. Positive (+4) and negative control were used.

Results: Of these samples, 9908 (23.6%) were ANA-IIF-positive. Totally 168 samples were considered as rare autoantibody. Rare patterns were consisted of 49 (0.49%) midbody, 29 (0.29%) centriole, 20 (0.20%) spindle fibers, 21 (0.21%) anti-golgi, 37 (0.37) anti-actin, 3 (0.03%) rods and rings, and 9 (0.09%) PCNA(proliferating cell nuclear antigen)-like. The number of females and males who have rare antibody was 101 and 67, respectively. All of the samples presented a fluorescence of ≥2+. There were 29 patients with systemic autoimmune diseases (SAID) from the rheumatology department. Another 139 patients were from gastroenterology, endocrinology, neurology and general internal medicine departments.

Conclusion: This article shared 6-year experience associated with rare ANA patterns. The significance of our results also emanates from the fact that they document a tertiary hospital's epidemiological data in Turkey.

Key words: Antinuclear antibody; rare autoantibody; immunofluorescence pattern

Introduction

Antinuclear antibody (ANA) can be helper to the diagnosis of systemic autoimmune diseases (SAID) and to monitor disease activity. Detection of ANA is essential to sub-classify patients with autoimmune disease. Although some ANA patterns have been comprehensively studied such as homogenous, nuclear or nucleolar staining patterns, rare antinuclear antibody patterns still require further assessment. Major limitations are the low frequency of the patterns, and circumscribed reports. Rare ANA patterns can be associated with some non-autoimmune conditions or SAID (1). This article seeks to scrutinize the studies and results associated with the prevalence and clinical significance of rare ANA patterns. Previously, our laboratory reported articles focusing on 4-year ANA results (2), and frequency of dense fine speckled pattern (3). Nonetheless, in our laboratory, rare ANA patterns have not been screened before. This article documents 6-year observation about rare ANA patterns in a medical microbiology laboratory of a tertiary hospital in Turkey. Evaluation of rare ANA patterns may be helper to clinical diagnosis. In laboratory practice, rare patterns should be an important part of microscopic evaluation. In this study, the rare pattern was defined as 1% occurring ratio on indirect immunofluorescence assay (IIF). The research was performed according to the World Medical Association Declaration of Helsinki.

Material and Methods

This study includes 41921 serum samples that different departments had sent to the tertiary Hospital's Laboratory of Medical Microbiology for ANA test between January 2010 and December 2015. In the study, first samples were included for each patient. Serum samples were studied in dilution of

1:100 using HEp-20–10/liver biochip (Monkey) and conjugated with specific antihuman IgG (Euroimmun AG, Lubeck, Germany). The fluorescence intensity was evaluated at x400 by immunofluorescence microscope (Eurostar III plus). Evaluation was performed as semi-quantitatively from 1+ to 4+. Positive (+4) and negative control were used. All of the slides were evaluated by same specialist. Clinical data were collected from medical records. IBM SPSS Statistics software version 24.0 and independent sample t test were used in statistical analysis.

Results

Of these samples, 9908 (23.6%) were ANA-IIF-positive. Totally 168 samples were considered as rare autoantibody. Rare patterns were consisted of 49 (0.49%) midbody, 29 (0.29%) centriole, 20 (0.20%) spindle fibers, 21 (0.21%) anti-golgi, 37 (0.37%) anti-actin, 3 (0.03%) rod and rings, and 9 (0.09%) PCNA (proliferating cell nuclear antigen)-like (Table 1). All of the samples presented a fluorescence of ≥2+. There were 29 patients with SAID and other diagnosis from the rheumatology department. SAID contains systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease (MCTD) and Sjögren's syndrome (SjS). Another 139 patients were from gastroenterology, endocrinology, neurology, infectious diseases, ophthalmology and general internal medicine departments (Table 2). The analyzed 168 patients with rare ANA included 107 females and 61 males. The mean age was 56.74±8.09 in females and 59.00±5.38 in males. The mean age of female cases was smaller than that of male cases and it was statistically significant ($p<0.05$).

Table 1. IIF patterns and diagnosis

IIF Pattern	n	%	Diagnosis	n
Midbody	49	0.49	Cancer (esophageal, gastric, colon)	22
			SAID	12
			Polyneuropathy	3
			Infectious disease	3
			Postmenopausal osteoporosis	1
			Other pathologies	8
Anti-actin	37	0.37	Autoimmune hepatitis/Primary biliary cirrhosis/Chronic liver disease	36
			Celiac disease	1
Centriole	29	0.29	SAID	14
			Cerebrovascular disease	8
			Other pathologies	7
Anti-golgi	21	0.21	SAID	2
			Chronic liver disease	9
			Infectious disease	4
			Other pathologies	6
Spindle fibers	20	0.20	Infectious disease	5
			Cancer	3
			Vasculitis	2
			Diabetes mellitus type 2	2
			Autoimmune hepatitis	1
			Other pathologies	7
PCNA-like	9	0.09	Infectious disease	2
			Polyneuropathy	2
			SAID	1
			Other pathologies	4
Rods and rings	3	0.03	Chronic hepatitis C	3

SAID: Systemic autoimmune diseases.
PCNA: Proliferating cell nuclear antigen.

Table 2. Diagnosis and departments of the patients that have rare ANA pattern

Department	Pre-diagnosis/diagnosis
Rheumatology	SAID
	Postmenopausal osteoporosis
	Ankylosing spondylitis
Gastroenterology	Hepatic fibrosis
	Cholangitis
	Cancer (esophageal, gastric, colon)
	Chronic hepatitis
Endocrinology	Diabetes mellitus type 2
Neurology	Cerebrovascular disease
	Polyneuropathy
Infectious diseases	Viral infection
Ophthalmology	Keratitis
General internal medicine	Seronegative rheumatoid arthritis
	Pneumonia

SAID: Systemic autoimmune diseases.

Discussion

Rare autoantibody patterns were evaluated as mitotic (midbody, spindle fibers, centriole and rod and rings), nuclear (PCNA-like) and cytoplasmic (anti-golgi, anti-actin) in our retrospective analyses. The midbody pattern was the most prevalent autoantibody in our study. The midbody is occurred in the final phase of cell division and it includes microtubuli related to the spindle mid-zone and certain associated proteins (1, 4, 5). Fang et al. (6) have investigated autoantibodies in the serum samples of primary hepatocarcinoma patients. They informed that the positivity rate of autoantibodies was 27.3% (38/139) in 139 patients. Anti-midbody antibody was detected in 1 patient (1/38, 2.6%). Vermeersch and Bossuyt (1) have informed that an unclear nuclear speckled staining is observable in interphase cells. In addition, this pattern is related with SjS, Raynaud's syndrome and cancer. In their retrospective analysis, they have reported 12 patients with midbody pattern and 5 patients had cancer. In our evaluation, 22 of 49 patients who have midbody pattern were gastrointestinal system cancer such as esophageal, gastric or colon.

Smooth muscle antibodies (SMA) and antinuclear antibodies are significant markers in the serological diagnosis of autoimmune hepatitis type 1 (AIH-1). SMA staining patterns for AIH-1 correlate with filamentous actin (F-actin) (7). A lot of articles have been published associated with anti-actin antibodies in AIH-1 patients (8, 9, 10). In our analysis clinical diagnosis of 36 patients were associated with liver diseases.

In animal cells, there is an essential microtubule organizing center during interphase and mitosis. It is composed of centrosome matrix or pericentriolar material. The structure is termed as centrosome (1, 11). The centrioles are part of centrosome. Anti-centriole antibodies have been observed rarely and reported

in patients with different systemic autoimmune disorders such as SjS, SLE, SSc. It may be seen inthe patients with viral or mycoplasmal infections (12). In Vermeersch P et al.'s article (1), the anti-centriole pattern was the rarest autoantibody pattern. Our results were different from the investigation in terms of case count. They reported anti-centriole antibody for six patients in 12-year period whereas we report 29 patients in 6-year period. This difference can be attributed to variations in Hep-2 cell series in conjunction with improvements in diagnosis technology and capabilities. In addition, epidemiological differences including patient population and geographical location might have played significant role in leading the difference in our results. However, Hamaguchi et al. (13) informed 5 scleroderma patients with anti-centriole antibody. They emphasized that anti-centrioleantibodies may help the diagnosis ofpulmonary arterial hypertension and digital ulcers or gangrene. Terreri et al. (14) have reported a case. They observed anti-centriole autoantibodies in a 49-year-old patient with acute thromboangiitis obliterans, several vascular risk factors and associated features of collagen vascular disease. We detected 8 patients with cerebrovascular disease in our analysis.

Rodriguez et al. (15) first reported anti-golgi antibodies in a patient with Sjögren's syndrome and lymphoma. Anti-golgi antibodies give a characteristic speckled staining on IIF comprised of irregular granules contiguous to one side of the nucleus (11). A lot of investigators reported cases with anti-golgi antibody that have autoimmune disease (15, 16, 17). However, Hong et al. (18) informed that most of the anti-golgi antibody-positive cases were found to be patients with non-autoimmune diseases (8/12 patients) in their evaluation. They emphasized that anti-golgi antibodies associated with clinical diseases require further assessment. In our evaluation there were 2 patients with SAID. There were additional antibodies such as Scl, SSA and SSB in immunoblotting assay in the patients. Similarly, Hattori et al. (19) reported a 74-year-old woman with anti-golgi antibody and anti-SS-A/Ro antibody who contracted inflammatory myopathy. Additionally, Mozo et al. (20) have firstly described a patient with hepatitis C virus-induced hepatocellular carcinoma, who has high titres of anti-golgi antibodies. There were 9 similar patients with chronic liver disease due to hepatitis B or C virus in the current study.

Mitotic apparatus proteins are responsible for specific functions during mitosis and the post-mitotic period. Mitotic apparatus is comprised of some structures such as centrosomes, spindle poles, spindle microtubules, and chromosomes and intercellular bridge (11). Bonaci-Nikolic B et al. (21) reported that the Nuclear-Mitotic Apparatus protein (NuMa-1) is responsible for organization of the mitotic spindle and the major target for mitotic apparatus antigens. Since both nucleoplasmic and mitotic spindle poles are stained, NuMA might be admitted as a "composite" pattern (11, 22). In an article (21), it has been informed that anti-mitotic apparatus antibody is one of the rare autoantibodies in patients who have symptoms related to autoimmunity. The importance of anti-mitotic apparatus antibodies should be evaluated together with clinical presentation and other systemic autoantibodies. Vermeersch P et al. (1) reported the 66 patients who have positive for anti-NuMa1 antibodies in their study. According to the analysis there were only 6 patients with systemic autoimmune disease: SLE (3 patients), SjS (1 patient) and undifferentiated connective tissue disorder (UCTD) (2 patients). Five patients were with rheumatoid arthritis. The authors informed that carcinoma was the most frequent non-autoimmune disorder related with anti-mitotic spindle apparatus antibodies. In our analysis, most of the patients that have spindle fibers pattern were from infectious diseases department. Notwithstanding, other reasons such as postmenopausal osteoporosis, cerebrovascular disease, fibromyalgia and osteoarthritis were also detected.

Miyachi et al. (23) firstly described antibodies to PCNA in some patients with SLE. Some authors have informed that anti-PCNA antibodies are rare but highly specific for SLE (24, 25). Beyne-Rauzy et al. (24) analyzed 8259 ANA tests between 1995 and 2000. They have reported that 12 patients were found positive for anti-PCNAs. The diagnosis of 12 patients was SLE. It has been informed that anti-PCNA antibodies may be found in more severe clinical signs (24) such as nephritis (26). As distinct from this analysis there was 1 patient, who has anti-PCNA-like, with SLE in our study. Other pathologies were gonarthrosis (2 patients), respiratory disease (1 patient) and atopic dermatitis (1 patient).

Rods and rings pattern is observed in chronic hepatitis C patients. It is occurred by ~3–10 μm rods and 2–5 μm rings on Hep-2 cells (11). However, researchers informed that rods and rings autoantibody positive cases are mostly received interferon treatment. Rods and rings antibody-positive patients give weaker response to therapy than the others (27). Conversely, Carcamo et al. (28) reported 8/23 persons who had rods and rings antibody without prior HCV infection. Although the prevalence was notably low, the primary reason for rods and rings antibody was found to be ribavarin and interferon combination therapy (29). In this study there were 3 patients that rods and rings antibody-positive and all of them were treated with ribavirin and interferon therapy.

Conclusion

This article shared 6-year experience associated with rare autoantibody patterns. It can be thought that rare ANA patterns do not have clinical importance; however, it should be considered with clinical and laboratory findings. In addition, our results showed that some rare ANA patterns, particularly mitotic patterns may be associated with non autoimmune conditions. The limitation of our analysis is the lack of detailed clinical findings of the patients. Notwithstanding, our laboratory is in a hospital which admits patients from Turkey's different regions. Therefore, this study's retrospective evaluation is worthy of attention. The significance of our results also emanates from the fact that they document a tertiary hospital's epidemiological data in Turkey.

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Author Contributions: Evaluation of the patterns, data collection and writing –A.G.S.

Conflict of Interest: No conflict of interest was declared by the author.

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