Research article

Antinuclear antibodies staining patterns and their clinical association in systemic lupus erythematosus: a cohort of 126 Libyan patients

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Abstract

Background: Nuclear constituent such as histone protein, double stranded (ds) DNA, DNA/histone complexes (nucleosomes), various nuclear enzymes and other protein/ribonucleoproteins are common target antigens for antinuclear antibodies (ANA). On this base, different intranuclear immunofluorescent ANA staining patterns can be detected in SLE.

Objective: To study a different ANA patterns in our patients with SLE and to detect the associations between ANA patterns and clinical manifestations of SLE in our patients.

Methods: The study included 126 SLE patients who were registered in rheumatology out patient's clinic in Tripoli Medical Center in Libya in the period from January 2015 to January 2018. All patients met ≥ 4/11 of the 1982 American College of Rheumatology (ACR-82) criteria. Age, sex and clinical manifestations of their illness at diagnosis were recorded. ANA analysis by immunofluorescence (IF) microscopy (HEP-2cells) was sent to Bioscientia laboratory Ingelheim, Germany for all patients. Anti double stranded DNA (crithidia luciliae) analysis was also sent to the same laboratory for all patients.

Results: One hundred and twenty six patients (124 females and 2 males) were included in the study. Their mean age was 32.46 years. The most common ANA pattern in our patients was combined homogenous and fine speckled (HS-ANA) which present in 50/126 (39.7%) of patients followed by speckled pattern (S-ANA) in 37/126 (29.4%) of patients. The classical homogeneous pattern (H-ANA) was present in only 13/126 (10.3%) of our patients. Nucleolar pattern (N-ANA) ± other patterns were recorded in 14/126 (11.1%). Clinical manifestations as renal disorder was associated with HS-ANA, H-ANA, and S-ANA patterns more than N-ANA± other patterns ($p<0.00001$). Arthritis was less associated with S-ANA pattern than other patterns (p - value=0.0049). Haematological disorders occurred more in H-ANA pattern than other patterns ($p<0.00001$). Positive anti dsDNA analysis was associated more with HS-ANA and H-ANA than N-ANA± other patterns and S-ANA ($p<0.0001$).

Conclusion: Combined homogenous and fine speckled (HS-ANA) is the dominant immunofluorescent antinuclear antibody pattern among Libyan patients with SLE which was more associated with renal disorders and positive anti dsDNA antibodies than other patterns. Nucleolar pattern ± other patterns were recorded in 11.1% of our patients and associated with least renal disorders and positive anti dsDNA antibodies.

Key words: Antinuclear antibodies, Systemic Lupus Erythematosus, Antids DNA antibodies.

Introduction

Systemic Lupus Erythematosus (SLE) is multisystemic, relapsing and remitting disease that involves multiple organs. Mucocutaneous, arthritis, serositis, haematological, renal, neurological and immunological disorders are the most common features which present in ACR82 criteria of SLE. Nuclear constituent such as histone protein, double stranded (ds) DNA, DNA/histone complexes (nucleosomes), various nuclear enzymes and other protein/ribonucleoproteins are common target antigens for antinuclear antibodies (ANA)[1].

On the basis of their different intranuclear distribution, immunofluorescent ANA staining patterns can be subdivided into homogenous / chromosomal (H-ANA), centromeric (C-ANA), speckled /extrachromosomal (S-ANA), nucleolar (N-ANA), nuclear membrane, nuclear dots and other defined patterns[2]. The use of immunofluorescent microscopy to identify ANAs was introduced by Holman, Kunkel and Friou already in the 1950s[3,4], and still remain the gold standard for ANA diagnostics[5,6].

ANA presence, its titer and pattern is very important for diagnosis of SLE and it is one of its criteria. But it is not related to SLE activity. Anti ds DNA antibody is also considered in ACR82 criteria of SLE and it is related to SLE activity.

Materials and Methods

The study included 126 SLE patients who were registered in rheumatology out patient's clinic in Tripoli Medical Center in Libya in the period from January 2015 to January 2018.

All patients met ≥ 4/11 of the 1982 American College of Rheumatology (ACR-82) criteria. Age, sex and clinical manifestations of their illness at diagnosis were recorded. ANA analysis by immunofluorescence (IF) microscopy (HEP-2cells) was sent to Bioscientia laboratory Ingelheim, Germany for all pateints. Anti double stranded DNA (crithidia luciliae) analysis was also sent to the same laboratory for all patients.

Statistical analysis: The mean age of patients and frequencies of different IF-ANA patterns were measured. Clinical and laboratory features frequencies for each ANA pattern groups were noticed. Differences in distribution of different staining patterns regarding clinical and laboratory features were analysed using Chi-square tests. All statistics were performed using SPSS V.20.0. For each statistical test, exact p values were reported.

Results

One hundred and twenty six patients (124 females and 2 males) were included in the study. Their mean age was 32.46 years. The most common ANA pattern in our patients was combined homogenous and fine speckled (HS-ANA) which present in 50/126 (39.7%) of patients followed by speckled pattern (S-ANA) in 37/126 (29.4%) of patients. The classical homogeneous pattern (H-ANA) pattern was present in only 13/126 (10.3%) of our patients. Nucleolar pattern (N-ANA) ± other patterns were recorded in 14/126 (11.1%). Other rare patterns as nuclear membrane and nuclear dots were present in 12/126 (9.5%) of patients. The first four pattern groups (HS-ANA, S-ANA, H-ANA & N-ANA) were considered large enough for statistical comparisons regarding different clinical features. Clinical manifestations as renal disorder was associated with HS-ANA, H-ANA, and S-ANA patterns more than N-ANA± other pattern ($p<0.00001$). Arthritis was less associated with S-ANA pattern than other patterns (p-value=0.0049). Haematological disorders occurred more in H-ANA pattern than other patterns ($p<0.00001$). Positive anti dsDNA analysis was associated more with HS-ANA and H-ANA than N-ANA± other pattern and S-ANA ($p<0.0001$).

Other clinical features (mucocutaneous, neurological disorder and serositis), no significant differences between the four groups was present (p=0.83, 0.40, 0.07) respectively.

Table 1: Shows the most common four ANA pattern groups and their clinical features frequencies in SLE patients

Clinical features	H-ANA (%)	S-ANA (%)	HS-ANA (%)	N-ANA (%)	P-value
Arthritis	62	43	64	64	0.0049
Serositis	23	11	16	0	0.073
Neurological	8	5	10	0	0.40
Mucocutaneous	77	81	76	79	0.833
Haematological	92	51	60	71	<0.00001
Renal disorder	31	27	38	7	<0.00001
Anti-dsDNA	69	30	74	43	<0.00001

Table 1 shows the most common four ANA pattern groups and their clinical features frequencies. The differences in clinical features between different ANA pattern groups were analysed using Chi-square test and p- values were measured.

Discussion

In our study, we made a comparison between the four common ANA patterns (HS-ANA, S-ANA, H-ANA and N-ANA) and we found a significant difference between them regarding arthritis, haematological, renal and positive anti dsDNA antibodies ($p<0.05$). No differences were found in other features (Serositis, mucocutaneous and neurological) $p>0.05$.

In a Swedish study by Frodlund *et al*[1], the most common ANA pattern was classical (homogenous) type followed by speckled (S-ANA), HS-ANA and N-ANA respectively.

They found a significant differences between the four groups regarding arthritis (p=0.02), neurological disorders (p=0.04) and positive anti dsDNA antibodies (p=0.001). No differences were found between other features (Serositis, mucocutaneous, haematological and renal disorders) $p>0.05$. These differences between our study and the Swedish study calls for further studies.

Nucleolar staining of antinuclear antibodies is not exclusive to patients suffering systemic sclerosis since it can occur in other autoimmune diseases, such as SLE[7]. The nucleolar ANA patterns present a low incidence in patients with SLE, with less than 9% reported in some studies[7]. The significant of nucleolar staining and antinucleolar antibodies in SLE is still unknown[7].

In our study, N-ANA pattern ± other patterns were present in 11.1% of patients and were associated with less renal disorders and positive anti dsDNA antibodies. But these results need more studies with larger group of N-ANA pattern.

Conclusion

Combined homogenous and fine speckled (HS-ANA) is the dominant immunofluorescent antinuclear antibody pattern among Libyan patients with SLE which was more

associated with renal disorders and positive anti dsDNA antibodies than other patterns.

Nucleolar pattern ± other patterns were recorded in 11.1% of our patients and associated with least renal disorders and positive anti dsDNA antibodies.

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