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Study of Determination of Ana Pattern, Distribution and Clinical Relationship

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Abstract:
Background: *Immune response directed against "self" is referred to as autoimmunity. When a patient presents with the signs and symptoms of an autoimmune disorder, a diagnostic work-up of many laboratory tests, including testing of anti-nuclear antibodies (ANA) are advised. ANA are the serological hallmark of autoimmunity. The antibodies that are created by the body against its own cell nuclei are called as anti-nuclear antibodies (ANA). Depending on the organelles and associated target antigens attacked, ANA produce various patterns on indirect immunofluorescence assay (IIFA) on a human laryngeal epithelial cell line (Hep-2 cell line). The present study, aims at analysing various ANA patterns identified and assessing their clinical significance in patients afflicted with autoimmune disorders.*
Methods: *In this observational study, 154 consecutive patient sera that tested positive for ANA, sent to the Department of Pathology, SVP IMSR Hospital, Ahmedabad over a period of 3 years from May 2019 to October 2021. HEp-2000® IgG fluorescent ANA-Ro test system kits by Immuno concepts is used in dilution of 1:80 to detect ANA by IIFA.*
Results: *Amongst the ANA positive patients, clinical features Arthralgia shows maximum prevalence comprising of 37.0% (n=57) and majority of patients diagnosed with other non-specific disorders, not defining AID. Amongst the AID, RA is the most common diagnosis, 17.5% (n=27) cases. Here, 6 types of ANA patterns have been identified in the 154 cases where speckled pattern 62.3% (n=96) with ANA grade 2 23.3% (n=36) cases is the most common finding.*
Conclusions: *Autoimmune disorders are chronic disorders with increased morbidity and mortality. Detection of ANA is important in determining the diagnosis and clinical progression.*
ANA detection by IIFA is the investigation of choice for detection of autoimmune diseases considering its clinical course.
KEY WORDS: *Anti-nuclear antibody, ANA, IIFA, Immunofluorescence.*

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I. Introduction

Immune response directed against "self" is referred to as autoimmunity. Autoimmunity was first conceptualized by Paul Erlich in 1908 and termed it as "horror autotoxicus". (1) The prevalence of autoimmune disorders (AID) in Western countries is of 3 to 5% with a considerable morbidity and mortality. However, the condition in Asian countries like India is still blurred because only a few studies on individual diseases have been undertaken.(2)

When a patient presents with the signs and symptoms of an autoimmune disorder, a diagnostic work-up of many laboratory tests, including testing of anti-nuclear antibodies (ANA) are advised. ANA are the serological hallmark of autoimmunity(3). ANA attack self-proteins within cell nucleus structures, which now also include autoantibodies against nuclear envelope components, mitotic spindle apparatus, cytosol, cytoplasmic organelles, and cell membranes.(4) Depending on the organelles and associated target antigens attacked, ANA produce various patterns on indirect immunofluorescence assay (IIFA) on a human laryngeal epithelial cell line (Hep-2 cell line)

Identification of ANA patterns and their target antigen on the basis of variable cellular staining pattern can be correlated to specific autoimmune disease and it helps in determining the clinical progression and overall disease activity. Their presence in serum may indicate either a systemic autoimmune disease like systemic lupus erythematosus (SLE), systemic sclerosis (SSc), multiple connective tissue disorder (MCTD), rheumatoid arthritis (RA), Sjogren's syndrome (Sjo S) and dermatomyositis (DM), or an organ-specific condition like lupus nephritis, autoimmune thyroiditis and hepatitis[3]. According to ICAP, ANA is majorly classified into 3 subtypes namely: nuclear, cytoplasmic and mitotic. In many cases, mixed patterns or overlap of different patterns may also be seen. The present study aims at analyzing various ANA patterns identified and assessing their clinical significance in patients afflicted with autoimmune disorders.

II. Objectives

To detect and identify ANA patterns by IIFA
To analyze various clinical conditions of ANA positive patients
To determine prevalence of various autoimmune disorders in ANA positive patients.

III. Methods And Materials

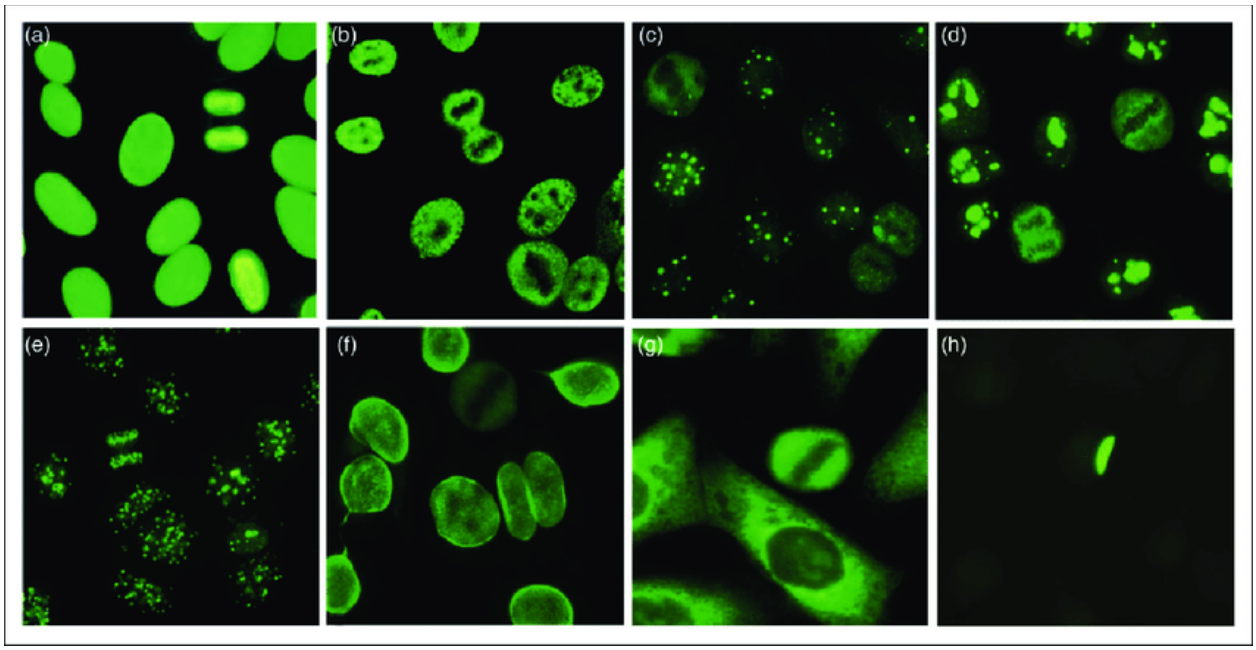
This is an observational study in which 154 consecutive patient sera that tested positive for ANA amongst the total ordered ANA tests, sent to the Department of Pathology, SVP IMSR Hospital, Ahmedabad over a period of 3 years from May 2019 to October 2021. HEp-2000® IgG fluorescent ANA-Ro test system kits by Immuno Concepts is used in dilution of 1:80 to detect ANA by IIFA.

ANA substrate slides use HEp-2000® cells (with mitotic figures) that are grown and stabilized directly on the test wells were evaluated under the fluorescence microscope equipped with 495 nm exciter filter and 515 nm barrier filter in a dark room using 40X objectives.

A) HEP-2000® IgG FLUORESCENT ANA-RO TEST SYSTEM KITS BY IMMUNO CONCEPTS
B) FLUOROSCENCE MICROSCOPE

Positive, negative, and PBS controls are tested one time per run. Fluorescence intensity was interpreted semi quantitatively based on the guidelines for fluorescent antibody reagents established by the Centers for Disease Control and Prevention, Atlanta, Georgia (CDC). negative control (0) and positive control (+4). This study was approved by the local ethics committee.

ANA PATTERNS



(a) Homogeneous, (b) speckled, (c) nuclear dots, (d) nucleolar (e) centromeres.
(f) nuclear membranes and (g) cytoplasmic (h) negative

INCLUSION CRITERIA:

1. Patient samples ordered for ANA tests and that were tested positive for the same have been included.

EXCLUSION CRITERIA:

1. Patient samples that were ordered for ANA test and were subsequently tested negative for ANA have been excluded from the study.
2. Insufficient quantity of sample
3. Hemolyzed sample
4. Lipemic sample
5. Samples containing precipitate or particulate matter or microbial growth
6. Samples collected with anticoagulant

IV. Observations And Results

From time period of May 2019 to October 2021 at a tertiary care hospital, Ahmedabad, a total of 154 patients were tested positive for ANA by IIFA.

Amongst the clinical features that are suggestive of AID, Arthralgia shows maximum prevalence comprising of 37.0% (n=57) cases, followed by fever comprising of 27.3% (n=42) cases.

Clinical features-wise distribution of patients

Clinical features suggestive of AID		
Clinical features of patients	**Frequency**	**Percentage (%)**
Fever	42	27.3
Arthralgia	57	37.0
Skin lesions	8	5.2
Raynaud's phenomenon	12	7.8
Systemic involvement	12	7.8

However, 42.2% patients show other clinical features, not suggestive of AID. Amongst those other clinical features, thrombocytopenia is the most common finding, present in 9.1% patients (n=10). Other findings

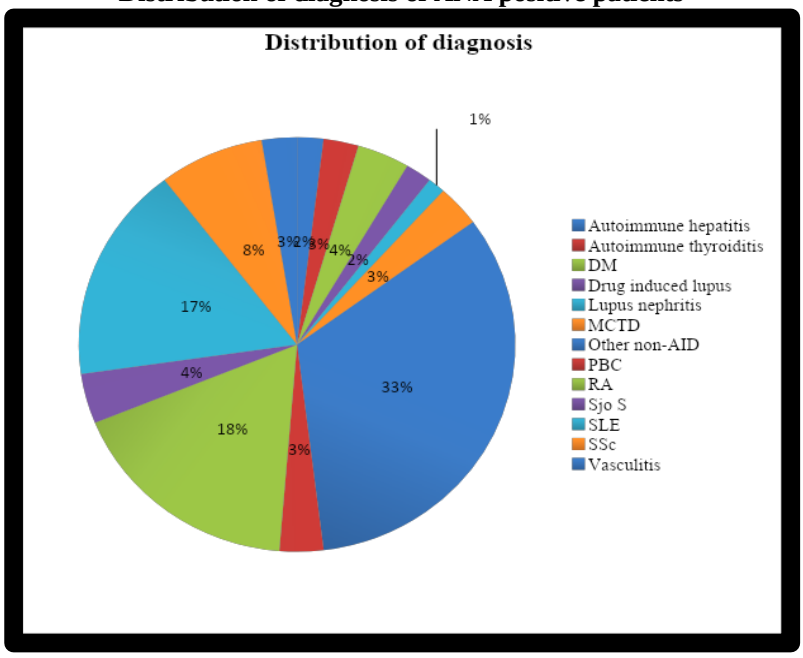
include COVID infection, ILD, CKD, TB and other non-specific findings.

The majority of patients that are tested positive for ANA are diagnosed with other non-specific disorders, not defining AID, comprising of 33.2% (n=51) cases. Amongst the AID, RA is the most common diagnosis, comprising of 17.5% (n=27) cases. It is followed by SLE comprising of 16.9% (n=26) cases.

Distribution of diagnosis of ANA positive patients

Diagnosis	Frequency	Percentage%
Autoimmune hepatitis	3	1.9
Autoimmune thyroiditis	4	2.6
DM	6	3.9
Drug induced lupus	3	1.9
Lupus nephritis	2	1.3
MCTD	5	3.2
Other non-AID	51	33.2
PBC	5	3.2
RA	27	17.5
Sjo S	6	3.9
SLE	26	16.9
SSc	12	7.9
Vasculitis	4	2.6
Total	**154**	**100**

Distribution of diagnosis of ANA positive patients



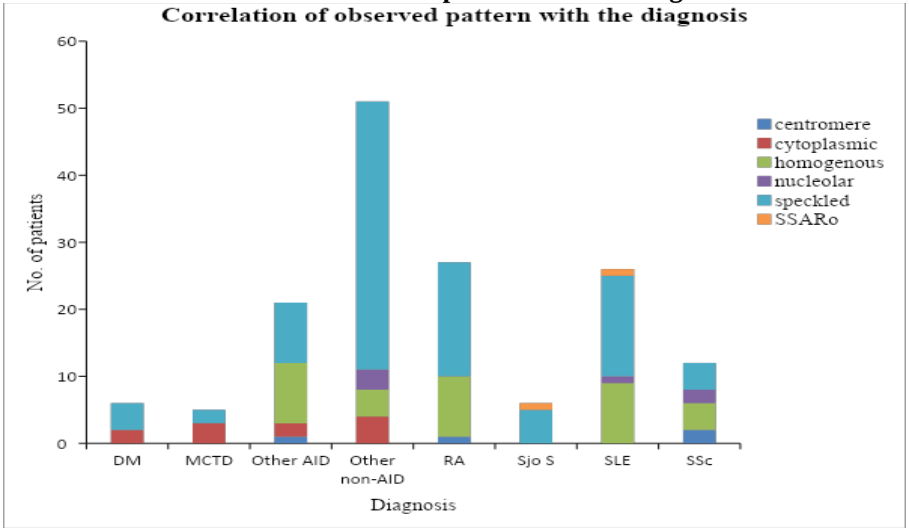
6 types of ANA patterns have been identified in the 154 cases. The speckled pattern is the most common finding, present in 62.3% (n=96) cases, followed by homogenous in 22.7% (n=35) cases. The least common ANA pattern identified is SSARo, present in 1.3% (n=2) cases.

The most common ANA grade is Grade 2 in speckled pattern comprising of 23.3% (n=36) cases.

Grade-wise distribution and correlation of observed ANA pattern with the diagnosis

ANA Patterns	Correlation with the diagnosis								Grade	Frequency	Percentage
	DM	MCTD	Other AID	Other non-AID	RA	Sjo S	SLE	SSc			
centromere	0	0	1	0	1	0	0	2	3	3	1.9
									4	1	0.7
cytoplasmic	2	3	2	4	0	0	0	0	2	5	3.2
									3	5	3.2
									4	1	0.7
homogenous	0	0	9	4	9	0	9	4	1	1	0.7
									2	10	6.5
									3	18	11.6
									4	6	3.9
nucleolar	0	0	0	3	0	0	1	2	1	2	1.3
									2	2	1.3
									3	1	0.7
									4	1	0.7
speckled	4	2	9	40	17	5	15	46.5	1	19	12.3
									2	36	23.3
									3	31	20.1
									4	10	6.5
SSA/Ro	0	0	0	0	0	1	1	0	2	1	0.7
									3	1	0.7
Total	**6**	**5**	**21**	**51**	**27**	**6**	**26**	**12**		**154**	**100.00**

Correlation of observed pattern with the diagnosis



V. Discussion

In our study, a total of 154 patients that tested positive for ANA by IIFA have been included.

In present study, amongst the clinical features that are suggestive of AID, Arthralgia shows maximum prevalence comprising of 37.0% (n=57) cases, followed by fever comprising of 27.3% (n=42) cases.

This correlated with the study by Mengeloglu et al[8] where joint pain was present in 26.0% (n=125) patients. It is also correlated with a study conducted by Kosaraju et al[9] and Angel et al[10]. Joint pain and fever are the most common clinical manifestation in the study conducted by Begum et al[11] which correlated with our study.

Comparison of most common ANA patterns across various studies

Study name	Most common ANA pattern	Prevalence (%)
Present study	Speckled (nuclear)	62.3
Tayde et al[67]	Speckled (nuclear)	56
Gupta et al[64]	Nuclear speckled	24.
Mengeloglu et al[65]	Nuclear speckled (coarse, fine, granular)	52.4
Sener et al[72]	Homogenous (nuclear)	18.7

Comparison of prevalence of ANA patterns and comparison of most common diagnosis across two studies

ANA pattern	Prevalence (%)		Most common diagnosis (Prevalence in the ANA pattern)	
	Present study	Mengeloglu et al[65]	Present study	Mengeloglu et al[65]
Centromere	2.6	4.6	SSc (50%)	Other unkown (56%)
Cytoplasmic	7.1	-	Other non-AID (36.3%)	-
Homogenous	22.7	4.0	RA (25.7%), SLE (25.7%), Other AID (25.7%)	Sjo S (65%)
Nucleolar	4.0	18	Other non-AID (50%)	Sjo S (86%)
Speckled	62.3	60.2	Other non-AID (41.6%)	Other unknown (49.8)
SSA/Ro	1.3	-	Sjo S (50%), SLE (50%)	-

6 types of ANA patterns have been identified in the 154 cases. The speckled pattern is the most common finding, present in 62.3% (n=96) cases, followed by homogenous in 22.7% (n=35) cases.

This correlated with the study of Tayde et al[12] where speckled was 56% and homogenous was 32% and also with a study by Gupta et al[6] in which speckled pattern was most common (24.0%) followed by homogenous (21.02%).

In a study by Mengeloglu et al[8], the most common ANA pattern was Nuclear speckled (includes coarse, fine and granular) in 52.4% cases followed by nucleolar in 18.0% cases. Cytoplasmic pattern was present in 7.1% (n=11) cases while in a study by Angel et al[10] it showed cytoplasmic pattern in 17% cases.

Overall in our study, the most common diagnosis is other non-AID in 33.2%(n=51) followed by RA in 17.5% (n=27) and SLE in 16.9% (n=26). This correlated with a study by Mengeloglu et al, where ANA positive was studied across various diseases. In that study, 73.0% (n=361) patients had other unknown diseases, not specifying any autoimmune diseases. Amongst them, ANA positivity was reported to be found in 5% of healthy individuals due to unknown reasons. Amongst the autoimmune disorders, the ANA positivity was most commonly determined in RA (8.5%, n=42), followed by SLE (5.9%, n=29), and rheumatoid vasculitis (5.7%, n=28) in the study by Mengeloglu et al.

In a study by Angel et al[10], the most common disease was SLE (37%) followed by MCTD (18%).

In our study, majority of patients were diagnosed for other non-AID. Most common ANA pattern in those patients is speckled. This suggests that speckled pattern may be present in presumably healthy patients, or patients with subclinical autoimmunity. In a study by Marin et al, three groups of putatively normal population was studied in which 13.4% patients were tested positive for ANA by IIFA at 1:80 titre. The most common ANA pattern was speckled (50.3%).

Tan et al[6] detected ANA positivity rate as 31.7% in healthy individuals using a dilution of 1:40 of the sera. It is suggested that test should be repeated in a patient that were positive for ANA after a 3-6 months period when the symptoms of the disease disappear. A clinical diagnosis maybe supported if the repeat test is positive; however a negative result suggests that the first positivity might be due to polyclonal B activation.

Given below is a comparison between our study and a study by Mengeloglu et al in the ANA patterns and its respective most common diseases along with their prevalence for the respective pattern.

VI. Conclusion

Autoimmune disorders are chronic disorders with increased morbidity and mortality. Detection of ANA is important in determining the diagnosis and clinical progression.

Majority of patients with ANA positive were diagnosed with diseases other than autoimmune disorders. This suggests that ANA maybe positive in various other conditions and maybe responsible for causing subclinical autoimmunity without causing rheumatoid or connective tissue or other autoimmune disorders. Hence, presence of ANA should be always correlated clinically.

However, amongst the autoimmune disorders, RA and SLE were the most common diagnosis in ANA patients. Majority of patients of RA showed speckled pattern followed by homogenous as compared to other patterns. Similarly, patients of patients of RA showed speckled pattern followed by homogenous as compared to other patterns. Equal patients of SSc showed positivity for speckled and homogenous pattern. MCTD patients predominantly showed cytoplasmic pattern. Thus, it suggests that various ANA patterns show predisposition to certain AID.

To conclude, ANA detection by IIFA is the investigation of choice for detection of autoimmune diseases considering its clinical course.

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