Prevalence of antinuclear antibodies in central Madhya Pradesh- A prospective study

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Abstract

Introduction: Autoimmune diseases (AID) are a manifestation of a self damaging immune response of the body to its own antigen. In any suspected AID, the first test to be done is ANA detection by indirect immunofluoresence using HeP 2 cells due to its sensitivity. A positive ANA test is further evaluated by performing immunoblot test to detect specific antigens causing autoimmunity. The present study was undertaken to study the prevalence of antinuclear antibodies in patient population of Central Madhya Pradesh as well as to study the prevalence of disease specific antigens.

Materials and Methods: A total of 650 patients of all ages and both sexes coming to our diagnostic centre between January to June 2018 for ANA testing were included in the study. Serum ANA was determined by indirect immunofluorescence (IIFA) using Hep 2 cell lines (Euroimmun, Germany). A positive result by IIFA was further tested by immunoblot method (Euroimmun, Germany) when requested by the clinician.

Result: Out of 650 patients ANA screen was positive in 280 (43.08%) patients and negative in 209(32.15%) patients. ANA blot was requested in 161 patients (57.5%) of the total 280 ANA screen positive patients. Out of the 161 patients, 69 had a positive Immunoblot test for one or more antigens. Total 53.69% patients were positive by both ANA screen and immunoblot test. The total coincidence rate was 44.5% between ANA screen and ANA blot test with a positive coincidence rate of 81.6% and negative coincidence rate of 79.1%.

Conclusion: ANA IIF is considered to be the gold standard screening test for detecting autoantibodies and ANA blot is done as a confirmatory test to detect specific autoantibodies against antigens causing autoimmunity. The combination of ANA IIF and ANA blot is an effective method to diagnose autoimmune diseases. If used alone, a possibility of missing a diagnosis is high and so a combinbation of these two tests is of great benefit to both the clinicians as well as laboratorians.

Keywords: ANA, IIF, ANA blot, AID.

Introduction

Autoimmune diseases (AID) are a manifestation of a self damaging immune response of the body to its own antigen and AID cause a significant health burden in 3-9% of the general population. It results in a spectrum of disease conditions and was described by Paul Ehlrich in the beginning of the 20th century as "horror autotoxicus" for the harmful effects caused by AID.

The effects of autoimmunity range from physiologic self reactivity required for normal immune system homeostasis to intermediate level of autoantibody production which do not have any clinical symptoms to more severe form of AID where autoantibodies cause major harm to the body.[1] The severity and susceptibility to AID is due to environmental and genetic factors such as major histocompatibility complex (MHC) where differences in single amino acid alleles affect peptide binding, thereby causing autoantigen presentation.[2] The diagnosis of AIDs is based on the presenting clinical symptoms as well as detection of antinuclear antibodies (ANA) by immunological methods in the serum of patients and also disease specific antibodies.[3] In any suspected AID, the first test to be done is ANA detection by indirect immunofluoresence using HeP 2 cells due to its sensitivity.[4] A positive ANA test is further evaluated by performing immunoblot test to detect specific antigens causing autoimmunity.[5] These tests require knowledge of the classification criteria of each disease so that a correct diagnosis is made.[6,7]

ANA can be produced by the body due to several environmental factors in healthy individuals such as during pregnancy, due to increasing age, family history of AID and also in cardiovascular diseases, infections or in malignancy.[8-12]

The present study was undertaken to study the prevalence of antinuclear antibodies in patient population of Central Madhya Pradesh as well as to study the prevalence of disease specific antigens.

Materials and Methods

A total of 650 patients of all ages and both sexes coming to our diagnostic centre between January to June 2018 for ANA testing were included in the study. The patients were divided into < 20, 21-40, 41-60, 61-80 and > 80 years age group. Serum ANA was determined by indirect immunofluorescence (IIFA) using Hep 2 cell lines (Euroimmun, Germany) at an initial dilution of 1:100 according to instructions

provided by the manufacturer. When a positive result was obtained, further dilutions were made. A positive result by IIFA was further tested by immunoblot method (Euroimmun, Germany) when requested by the clinician and results were read by Euroline scan software (Euroimmun) for antibodies against nRNP, Sm, SS-A, Ro 52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, Nucleosomes, Histones, Rib P Protein and AMA M2. The positive ANA result was correlated with positive immunoblot and a coincidence rate was calculated from the data obtained.

Results

A total of 650 patients of all ages and both sexes were included in the study. There were 142 males (21.85%) and 508 (78.15%) females. The male to female ratio was 0.27:1. Maximum patients were in 41-60 years age group (33.08%), followed by 30.77% in 21-40 years, 8.31% in 61-80 years and 6% below 20 years of age. There were no patients above 80 years of age (Table 1).

Out of 650 patients ANA screen was positive in 280 (43.08%) patients and negative in 209(32.15%) patients. ANA blot was requested in 161 patients (57.5%) of the total 280 ANA screen positive patients. Out of the 161 patients, 69 had a positive Immunoblot test for one or more antigens. Total 53.69% patients were positive by both ANA screen and immunoblot test. The total coincidence rate was 44.5% between ANA screen and ANA blot test with a positive coincidence rate of 81.6% and negative coincidence rate of 79.1%. (Table 2)

In the ANA screen test speckled nuclear pattern was the most common finding accounting for 17.85% of all ANA screen positive cases, followed by homogeneous pattern (8.59%), speckled with cytoplasmic (5.05%), cytoplasmic (5%), mixed cytoplasmic (4.55%), nucleolar (1.85%), homogeneous with cytoplasmic (1.68%) homogeneous with nucleolar (1.01%). There was one case each of cytoplasmic with lysosomal pattern, speckled with spindle, speckled with nucleolar, homogeneous with granular and centriole pattern (0.17%). (Table 3)

Among the positive ANA blot patterns SSA/Ro60 was the most common (6.21%), followed by SSA/Ro 52 (5.59%), dsDNA and histones 3.73% each, SSB/la and nucleosomes 3.11%) each, PCNA, Ribosomal-Po, Scl 70, U1SnRNP AND Jo1 2.48% each, SmD1 (1.86%), CeNP-B (1.24%) and AMA M2, PMScl and Mi2 (0.62%) each. (Table 4)

Table 1: Demographic data of patients

S. No	Age	Male		Female	
		Total	%	Total	%
1	< 20	24	3.69%	39	6.00%
2	21 - 40	46	7.08%	200	30.77%
3	41 - 60	57	8.77%	215	33.08%
4	61 - 80	15	2.31%	54	8.31%
	Total	142	21.85%	508	78.15%

Table 2: showing distribution of ANA and ANA blot

	Positive	%	Negative	%
ANA	280	43.08%	209	32.15%
ENA	69	10.62%	92	14.15%
Total	349	53.69%	301	46.31%

Table 3: Patterns of positivity in ANA

S. No	Pattern of ANA	Total	%
1	Homogenous	51	8.59%
2	Homogenous + Coarse Granules	2	0.34%
3	Homogenous+Nucleolar	6	1.01%
4	Homogenous+Cytoplasmic	10	1.68%
5	Homogenous+Granular	1	0.17%
6	Homogenous+Speckled	2	0.34%
7	Speckled	106	17.85%
8	Speckled+Cytoplasmic	30	5.05%
9	Speckled+Nucleolar	1	0.17%
10	Speckled+Spindle	1	0.17%
11	Nucleolar	11	1.85%
12	Centromere	3	0.51%
13	Cytoplasmic	13	2.19%
14	Mixed Cytoplasmic	27	4.55%
15	Centriole	1	0.17%

16	Nuclear dots	3	0.51%
17	Nucleolar	11	1.85%
18	Cytoplasmic with lysosomal pattern	1	0.17%

Table 4: Patterns of positivity in ANABlot

S. No	Pattern of ANA	Total	%
1	SS-A/Ro60	10	6.21%
2	SS-A/Ro52	9	5.59%
3	SS-B/La	5	3.11%
4	dsDNA	6	3.73%
5	Histone	6	3.73%
6	Nucleosome	5	3.11%
7	PCNA	4	2.48%
8	Ribosomal-P0	4	2.48%
9	CENP-B (Centromere Protein B)	2	1.24%
10	Scl-70	4	2.48%
11	U1-snRNP	4	2.48%
12	AMA-M2	1	0.62%
13	SmD1	3	1.86%
14	Jo-1	4	2.48%
15	PM-SC1	1	0.62%
16	Mi-2	1	0.62%
17	Ku	0	0.00%

Table 5: Co-relation table including various single antigens with their immunofluorescence patterns & clinical associations

Location	Pattern	Target Antigen	Clinical Association
Nucleus	Homogeneous	Double Strand DNA Histones Nucleosome, RNA, Single Strand DNA	SLE Drug induced Lupus, SLE, RA SLE, MCTD, RA, RM, DM, SS
	Speckled	Sm U1-snRNP SSA/Ro SSB/La Ku Cyclin1(PCNA) Mitosin/Cyclin II	SLE MCTD, SLE, RA, sharp syndrome Sjogren's syndromes (SS)/SLE/Neonatal Lupus PM/DM/SLE/SS SLE/Overlap syndromes DM
	Dense fine speckled(DFS)	Lens epithelium-derived growth factor (LEDGF), DNA binding transcription coactivator p75.(DFS-70)	Healthy individuals, various inflammatory conditions like atopic dermatitis, interstitial cystitis, Asthma.
	Centomeres	Protein of Kinetochores	CREST syndrome, PSS limited form
	Nuclear Dots	Sp-100, NDP53	PBC, Rheumatic Disease
	Nuclear Membrane	Lamins, gp210, p62	CFS, Collagenoses, PBC, AIH
Nucleolus	Nucleolar homogeneous	PM-Scl Scl-70	PM, DM, PSS (Difusse) PSS (Difusse)
	Nucleolar Speckled	RNA-Polymerase I / NOR-90	Progessive systemic Sclerosis (Diffuse)
	Nucleolar Pattern	Fibrillarin	Progressive systemic Sclerosis(Diffuse)
Cytoplasm	Cytoplasmic Speckled	Mitochondrial Lysosomal Golgi Complex Ribosome P Jo-1 SRP, PL12, TIF1-Gamma	PBC. Unknown SS/SLE/RA SLE Polymyosotos (PM), PM/DM, Myositis.

	Cytoplasmic filament	F-Actin Vimentin Tropomyosin Cytoplasmic Rings & rods	AIH Unknown Unknown HCV Infection-on therapy
Cell Cycle (mitotic cells)	Centriole Mid-Body Spindle Fibres	--	Unknown Unknown Rheumatic Disease

Table 6: ANA blot profile pattern

Test	Disease Association
ds DNA	SLE (40-90%)
Nucleosome	SLE
Histones	Drug induced lupus (95%), Rheumatoid arthritis (15-50%)
SmD1	SLE
PCNA (Proliferating cell Nuclear Antigen)	SLE (3%)
Ribosomal-P0	SLE
SS-A/Ro60	Sjogren syndrome (40-80%), disseminated lupus erythematosus (30-40%), primary billiary cirrhosis (20%)
SS-A/Ro52	Sjogren syndrome
SS-B/La	Sjogren syndrome (40-80%), disseminated lupus erythematosus (10-20%)
CENP-B(Centromere Protein B)	CREST syndrome (70-90%)
Scl-70	Systemic sclerosis
U1-snRNP	MCTD, Sharp syndrome
AMA-M2	Primary biliary cirrhosis, Progressive systemic sclerosis
Jo-1	Polymyositis (25-35%), Interstital lung fibrosis
PM-SC1	Overlap syndrome(Polymyositis, dermatomyositis & Progressive systemic sclerosis

Discussion

Autoantibodies directed against the cellular components are found in the sera of patients having autoimmune diseases and are commonly detected in systemic diseases such as systemic lupus erythematosus (SLE), scleroderma, polymyositis and Dermatomyositis etc.[13] They are also detected in patients with specific organ related autoimmune diseases such as Hashimoto's thyroiditis, hepatitis etc and in certain malignancies and infections.[14] They may be present in individuals with no specific clinical symptoms.[14,15] The most common antibodies to be tested for diagnosing AID are antinuclear antibodies (ANA) by indirect immunofluorescence method which includes antibodies against both nuclear and cytoplasmic components of a cell.[16]

Out of 650 patients in our study, ANA was positive in 43.08% cases. The total coincidence rate between ANA and ANA blot was 44.5% with a positive and negative coincidence rate of 81.6% and 79.1% respectively. The females outnumbered the males in our study. Our study correlates with the study by Maria Elena et al who observed that 80% were females and 20% males in their study population.[17] In their study 52% were positive for ANA which is somewhat similar to our study. They observed that 52% patients positive for ANA had detectable levels of specific antibodies by Immunoblot. We observed a lower incidence of Immunoblot positivity probably because not all ANA positive patients were tested for specific antibodies. It may also be due to the fact that healthy subjects and people with diseases other than due to auoimmunity or with a family history of AID may have low titres of ANA in their sera.[18,19]

Racoubian et al in their study observed ANA positivity of 26.4% in 10851 subjects with 3311 males and 7503 females.[20] Sebastian et al in their study in South Indian population observed 45.5% dsDNA positivity as also for nucleosomes, histones SSA/Ro52.[21] Ya Ping Guo et al in their study of Chinese population observed an overall ANA prevalence of 5.92% and when correlated with ANA blot, they observed that 44.2% were positive for atleast one of the 15 antigens tested.[22] Minrou Satoh et al in their study observed ANA prevalence rate of 13.8% in their population with a higher incidence in females as compared to males.[23] Ciu Kong et al observed a total consistent rate between ANA and ANA blot to be 86.7% with a positive and negative consistency rate of 61.1% and 95.2% respectively.[24] Our study differs from this study as we observed a total consistency rate of 44.5% with a positive and negative consistency rate of 81.6% and 79.1% respectively. This inconsistency between different studies may be due to limitations of the different methodologies used. In IIFA low concentration of autoantibodies may be missed due to destruction of antigens during preparation of Hep2 substrate resulting in negative ANA.[25] Moreover, ANA positivity differs in different geographical locations. It may also be due to presence of antibodies other than those being tested resulting in negative ANA blot in spite of positive ANA screen.[26-28]

Our study has certain limitations. Not all antibodies were tested which may cause autoimmune diseases as also that many non AIDs may also cause ANA positivity. Moreover, low titres of antibodies may have been missed.

Conclusion

ANA IIF is considered to be the gold standard screening test for detecting autoantibodies and ANA blot is done as a confirmatory test to detect specific autoantibodies against antigens causing autoimmunity. The combination of ANA IIF and ANA blot is an effective method to diagnose autoimmune diseases. If used alone, a possibility of missing a diagnosis is high and so a combination of these two tests is of great benefit to both the clinicians as well as laboratorians.

Conflict of Interest: None

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