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Detection and identification of antinuclear antibodies (ANA) in consecutive serum samples for routine autoantibody testing referred for ANA testing

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Abstract:

Studies on autoimmune diseases in India are very less. There are scanty of research work done and data available on this topic in India. In this project work, retrospective study on data from January 2012 to December 2013 and prospective study from January 2014 to May 2014 has been done. In autoimmune illnesses, the existence, type, and strength of an autoimmune response may be gleaned from the serum auto-antibodies that are detected during diagnosis. 1441 consecutive patients (881 Female and 560 male) were evaluated prospectively. In the present study 330 (22.90%) patients were found positive for ANA and the percentage of ANA positive was found more in female 73% (241) then male 26.96%. Majority of cases showed speckled pattern 33.94% (112) followed by homogenous 26.66% (88), nucleolar 10.90% (36), cytoplasmic 2.12% (7), and 9.69% (32) showed mixed pattern.

1. Introduction:

The presence of antinuclear antibodies (ANA) is demonstrative of immune system connective tissue sickness. Anti-nucleic acid antibodies (ANAs) tie to cell proteins, nucleic acids, and nucleic corrosive protein buildings [1]. Conclusion of immune system connective tissue problems such polymyositis/dermatomyositis, systemic lupus erythematosus (SLE), and Sjogren's condition has depended on ANA recognition from its most memorable distribution in 1948 [2]. People with

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connective tissue diseases often have higher titers of ANAs than the normal population (20-30%) [3]. Therefore, the diagnostic value of ANA detection methods depends on their sensitivity and specificity**.** With the help of the cell's nucleus, ANAs bind to numerous molecules, including proteins and nucleic material. Studies indicate that antibodies are created when cells undergo incomplete apoptosis, which results in the formation of double-stranded DNA that can bind to antibodies (anti-dsDNA) [4,5]. The Smith protein, which is found inside small nuclear ribonucleoprotein (snRNP) particles, is also bound by anti-Sm antibodies. Antibodies to Scl-70 bind to Topoisomerase I, preventing DNA replication, and antibodies to anti-centromere attach to interphase centromeres, preventing cell division [6,7,8].

In 1948, Hargraves and collaborators distributed the main record of the improvement of ANA testing when they distinguished an exceptional cell in the body of a patient with basic lupus erythematosus (SLE) and named it the "L.E. cell" [9]. The most broadly perceived technique for ANA revelation incorporates the participation of patient blood antibodies with fixed HEp-2 cells to create novel fluorescent models [10].Strategic, HEp-2 planning, and neutralizer articulation heterogeneity might make normalization of HEp-2 IIF information troublesome [11]. Immunizer business boards for SSA/Ro, SSB/La, Sm, Scl-70, Jo-1, and centromeres may likewise be bought. ELISA has shown equivalent performance to IIF [12] and allows for quantitative screening of specific ANAs. These benefits are also shared by multiplex immunoassays, which utilise a combination of several types of recognized antigen-coated beads. They prove their worth when combined with patient serum [13].

2. Material and Method:

2.1 Sample Collection:

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Blood sample that has already been collected were centrifuged to collect serum of the patients and further examined. Samples consisted of 1441 ANA, 189 ENA, 155 Anti – DNA, 147 ALP, 279 ANCA consecutive serum samples for routine autoantibody testing.

2.2 Instruments Used:

Micropipette, Centrifuge, lass BIOCHIPs slides, Fluorescent Microscope, LAF, Sterile Water, Sterile Accessories and etc.

2.3 Chemicals Used:

This kit contains slides each containing 5 x 2 BIOCHIPs coated with the substrate HEp-20-10 cells and primate liver, conjugate fluorescein-labeled anti-human IgG (goat), positive control against cell nuclei (ANA), negative control, buffer PBS (Phosphate buffer saline), Tween 20, glycerol embedding medium, cover glass.

2.4 Procedure:

BIOCHIP slides were hatched with 30 µl of debilitated model serum in each reaction field of the reagent plate at room temperature (+18°C to +25°C) for 30 minutes preceding being flushed with PBS-Tween for 5 minutes and 25 µl of fluorescein named foe of human globulin was added. slides from the BIOCIP were cleaned with PBS for 5 minutes at room temperature (18°C to 25°C).immediately after adding 10 µl of embedding medium and covering the slides with the cover slip. Slides are then observed under fluorescence microscope under 20x for tissue section, infected and transfected cells and under 40x for cell substrates. Clinical relevance and diagnostic accuracy of ANA subtypes To learn more, please see tables 01 and 02.

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Table No. 01 Evaluation of ANA and its therapeutically relevant subgroups for sensitivity a specificity.			
Autoantibodie	**Associated CTD**	**Sensitivity**	**Specificity**
ANA	SLE	93	57
	Sjogren's syndrome	48	52
	SS	85	54
	PM/dermatomyositis	61	63
	Raynaud phenomena	64	41
Specific ANA			
Anti-dsDNA	SLE	57	97
Anti-Sm	SLE	25–30	High*
Anti-SSA/Ro	Sjogren's syndrome, subacute cutaneous SLE, Neonatal lupus syndrome	8–70	87
Anti-SSB/La	Sjogren's syndrome, subacute cutaneous SLE, Neonatal lupus syndrome	16–40	94
Anti-U3-RNP	SS	12	96
Anticentromere	Limited cutaneous SS	65	99.9
Scl-70	SS	20	100
Jo-1	PM	30	95

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Table No. 02 Patterns of IF-ANA that are often seen in illnesses		
ANA pattern	**Antigen**	**Associated diseases**
Speckled	ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, 1, ribosomal-P	SLE, Mixed CTD, SS, Primary Sjogre syndrome, PM
Homogenous	dsDNA, Histones	SLE, Drug induced SLE
Peripheral (rim)	RNP, Sm, SSA/Ro	SLE, SS
Nucleolar	Anti-PM-Scl, anti-RNA polymerase I-III, a U3-RNP, To RNP	SS, PM
Centromere	CENP A-E	Limited SS

2.5 Statistical Analysis:

Patterns of the autoantibody tests, total patients, percentage of total positive patients, percentage of the positive diseased patients were analyzed. Gender wise prevalence of auto-antibodies were analyzed and bar graph prepared.

Ethics committee: All study procedures were approved by the local ethics committee.

3. Results and discussion:

There was a marked evaluation in IF pattern interpretation for auto-antibodies detection on HRp-2 cells in the last three years (2012, 2013 and 2014). According to the ANA result records, different possible patterns of fluorescence are described. 1441 consecutive patients (881 Female and 560 male) were evaluated prospectively. Refer table no. 04 and Graph no. 01. The majority of ANA-positive ARDs patients were female. Females had a greater rate of ARDs than males did (27.4%

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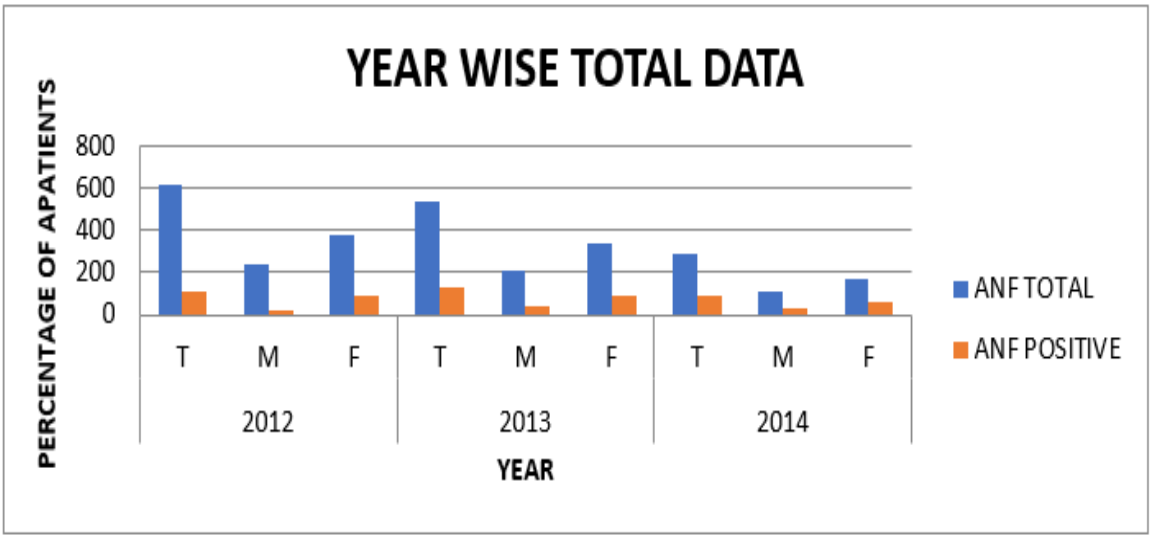
vs 16.0%), albeit this distinction was not genuinely huge. If it's not too much trouble, see Table 04 and Diagram 01 for subtleties.

Table No. 4 Year wise ANA total patients and positive patients.

TEST	2012			2013			2014		
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE
ANF TOTAL	619	240	379	539	206	333	283	114	169
ANF POSITIVE	108	19	89	132	38	94	90	32	58



Graph No 01: Year wise total ANA patients, total positive patients and male female.

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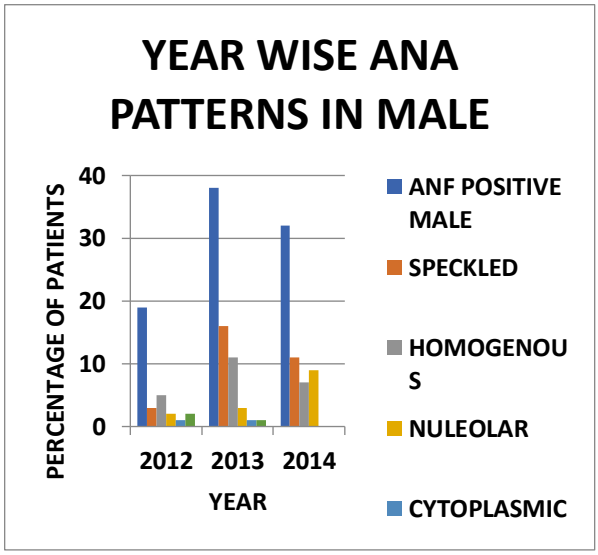
Three-year (2012, 2013 and 2014) ANA Male Patterns of test results are as – 69 Positive male patients, 30 male patients were having speckled pattern, 23 homogenous male patients, 14 nucleolar male patients, 7 cytoplasmic male pattern, 3 mixed pattern. Refer table no 05 and graph no. 02.

Table No. 05 Year wise ANA pattern in total male positive patients.

Year/Male	POSITIVE	SPECKLED	HOMOGENOUS	NULEOLAR	CYTOPLASMIC	MIXED
2012	19	3	5	2	1	2
2013	38	16	11	3	1	1
2014	32	11	7	9	0	0

Graph No. 02: Year wise ANA patterns in positive patients Male.



Three-year (2012,2013 and 2014) ANA Female Patterns of test results are as – 241 Positive female patients, 82 female patients were having speckled pattern, 65 homogenous female patients, 22 nucleolar female patients, 5 cytoplasmic female pattern, 27 mixed pattern. Refer table no 06 and graph no. 03.

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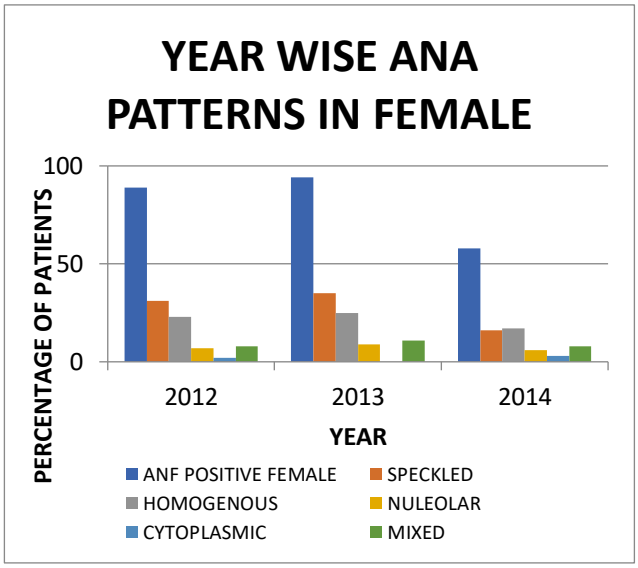
Table No. 06 Year wise ANA pattern in total female positive patients.

Year/ Female	POSITIVE	SPECKLED	HOMOGENOUS	NULEOLAR	CYTOPLASMIC	MIXED
2012	89	31	23	7	2	8
2013	94	35	25	9	0	11
2014	58	16	17	6	3	8

Graph No. 03: Year wise ANA patterns in female patients.



2012-year (Male & Female) Patterns of test results are as –619 total ANF Positive (Male & Female) patients, 108 (Male & Female) ANF positive patients, 34 (Male & Female) was having speckled pattern, 28 homogenous (Male & Female) patients, 09 nucleolar (Male & Female) patients, 03 cytoplasmic (Male & Female) pattern, 12 mixed pattern. Refer table no 07 and graph no. 04.

2013-year (Male & Female) Patterns of test results are as –539 total ANF Positive (Male & Female) patients, 132 (Male & Female) ANF positive patients, 51 (Male & Female) was having speckled pattern, 03 homogenous (Male & Female) patients, 12 nucleolar (Male & Female) patients, 01 cytoplasmic (Male & Female) pattern, 12 mixed pattern. Refer table no 07 and graph no. 04.

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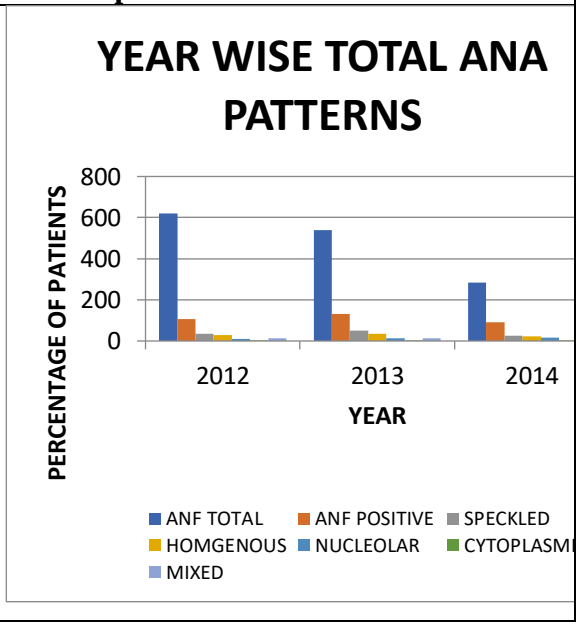
2014-year (Male & Female) Patterns of test results are as – 283 total ANF Positive (Male & Female) patients, 90 (Male & Female) ANF positive patients, 27 (Male & Female) was having speckled pattern, 24 homogenous (Male & Female) patients, 15 nucleolar (Male & Female) patients, 03 cytoplasmic (Male & Female) pattern, 08 mixed pattern. Refer table no 07 and graph no. 04.

Table No.07 Year wise ANA pattern in total positive patients

Year	ANF TOTAL	ANF POSITIVE	SPECKLED	HOMOGENOU	NULEOLAR	CYTOPLASMI	MIXED
2012	619	108	34	28	9	3	12
2013	539	132	51	3	12	1	12
2014	283	90	27	24	15	3	8

Graph No. 04 Year wise ANA patterns in male patients.



Three-year (2012, 2013 and 2014) ANA (Male & Female) Patterns of test results are as –1441 total ANF, 330 ANF Positive (Male & Female) patients, 112 (Male & Female) patients were having speckled pattern, 88 homogenous (Male & Female) patients, 36 nucleolar (Male & Female) patients, 7 cytoplasmic (Male & Female) pattern, 32 mixed pattern. Refer table no 08 and graph no. 05.

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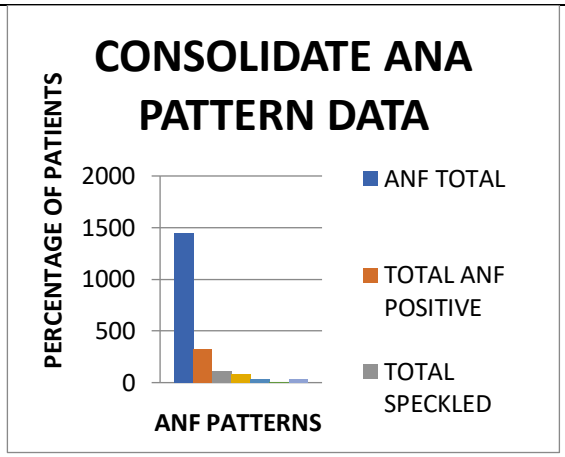
Table No. 08 Consolidate data of ANA patterns in positive patients.

PATTERNS	ANF TOTAL	ANF POSITIVE	Total SPECKLED	Total HOMOGENOUS	Total NULEOLAR	Total CYTOPLASMIC	Total MIXED
VALUES	1441	330	112	88	36	7	32

Graph No. 05: Consolidated ANA patterns of total positive



4. Conclusion:

When autoimmunity is suspected, a test for ANA IIFA should be performed. Therefore, it's safe to say that women are more likely to have an autoimmune disorder. However, why it is so could not be ruled out in the present study. Possible explanations include hormonal variances in women and men, or because females show increased immune reactivity or due to different levels of gender specific hormones and reproductive functions.

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