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THE PATTERN ANALYSES AND PREVALENCE OF ANTINUCLEAR ANTIBODIES IN THE GENERAL POPULATION OF THE STATE OF WEST BENGAL: A CROSS SECTIONAL STUDY

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ABSTRACT **INTRODUCTION :**Systemic Autoimmune Disease is a condition when our immune system attacks our own antigen; these antigens are diagnosed as foreign to our immunity system. The immune system normally protects our body from germs like bacteria & viruses. When our immune system senses our own proteins as foreign invaders, it sends out an army of fighting cells to attack them & release protein called autoantibodies that attack healthy cells. This autoantibodies or anti nuclear antibodies are hallmark of presence of Systemic Autoimmune Disease. **OBJECTIVES:** 1)To Estimate Auto Immune Connective Tissue Disease Burden in West Bengal2) Pattern Analysis of the Different Categories of Connective Tissue disease3) To find out the age distribution among the patients and patterns of diseases. **MATERIALS AND METHODS:** The serum samples which were positive for ANA by IIF method or those which were negative by IIF method but requested by the rheumatologists on clinical grounds were further processed for line immunoassay. Nylon strips coated with recombinant and purified antigens as discrete lines with plastic backing (EUROIMMUN AG) coated with antigens nRNP / Sm, Sm, SSA, Ro-52, SSB, Scl-70, PM-Scl, PCNA, Jo-1, CENP-B, dsDNA, nucleosomes, histones, ribosomal protein-P, anti-mitochondrial antibodies (AMA-M2) were used **RESULTS:** We have done a detailed analysis of different patterns found in ANA Hep -2 analyses. 44% tested positive sample are found to be associated with speckled (Coarse and fine) pattern. We have done a detailed analysis of different patterns found in ANA Hep -2 analyses. 44% tested positive sample are found to be associated with speckled (Coarse and fine) pattern. Immunoassay of 156 samples with ANA-IIF centromere, nucleolar and Nuclear Dots, Cytoplasm, Speckled& cytoplasm patterns reveal that Centromere patterns were observe in 9 samples (2.2%),7 samples shows positivity for line immunoassay(77.7%).samples shows positivity for CENP-B(57.14%)& specific combination of antigens RNP/Sm/SSA/RO-52(42.9%). **CONCLUSIONS:** The Pattern analysis clearly favoring a diagnosis of systemic sclerosis that too occurring an age group of 31-40 preponderance in male and equally distributed in all age groups among female in this geographical area. So if we can with the help of these wonderful cost friendly easy tests diagnose the sufferers at early stages of diseases we can thus help clinicians to make symptom free and healthy life span for this dreaded disease bearers.

KEYWORDS : Antinuclear Antibody Test, Immune Profile, Line Immunoassay

INTRODUCTION

Systemic Autoimmune Disease is a condition when our immune system attacks our own antigen; these antigens are diagnosed as foreign to our immunity system. The immune system normally protects our body from germs like bacteria & viruses. When our immune system senses our own proteins as foreign invaders, it sends out an army of fighting cells to attack them & release protein called autoantibodies that attack healthy cells. This autoantibodies or anti nuclear antibodies are hallmark of presence of Systemic Autoimmune Disease.

Due to high sensitivity & Specificity, Indirect Immunfluorecence (IIFT) using (Human epithelial cells & primate liver) is the gold standard for the detection of Anti Nuclear Antibody because here around 150 antigens are coated with tissues. This IIFT test used as screening test for identification of Autoimmune Diseases. If analysis is positive, further confirmed by defined single antigens(ELISA,Western Blot,Line blot immuneasssay) .[1-3]

These studies have been prepared or influenced by different articles of Western Countries but there are huge difference in immunity status, individual response to disease & type of antibodies, person to person, place to place &population to population. In this study, we are trying to reveal most common pattern in IIFT test in Kolkata (WB).This is a cross sectional study, analyzed random serum sample from population of West Bengal, referred to our Rheumatology Lab from different departments. This is the teaching hospital, providing service for ANA testing by IIFT & samples further processed for identification of specific antibodies by line immuneassay in that population & try to correlate between them [4]. After testing, if any correlation persists between two, one could restrict performing line immunoassay which is expensive & use ANA IIFT.This helps to reduce cost of laboratory investigation in developing the strategies to combat the diseases. [5]

OBJECTIVES

1) To Estimate Auto Immune Connective Tissue Disease Burden in West Bengal
2) Pattern Analysis of the Different Categories of Connective Tissue disease
3) To find out the age distribution among the patients and patterns of diseases

MATERIALS AND METHODS

Serum samples of patients from a random population of West Bengal who came to Hospital for medical help for treatment of rheumatic disease as suspected by rheumatologists / in-house specialists/ internal medicine specialists/dermatologists/ nephrologists or from any hospital department for a diagnosis of CTD were subjected for ANA testing by indirect immunofluorescence (IIF) method and line immunoassay during the study period of 12months (December 2017 to December 2018). Place of sample collection –Biochemistry Department, Medical College, Kolkata, (WB).

1024 Fresh whole blood samples were collected with patient consent at the phlebotomy section of Biochemistry department.t Serum separated from the clotted blood samples by centrifugation then it was stored at 4˚C if testing was planned within 72 hours or at -20˚C for testing after three days (without freezing and thawing). A fasting blood sample was most often used to avoid lipemic serum which could result in increased background fluorescence or unclear staining pattern. [6]

All serum samples, that selected for this study with a request for ANA by IIF method and line immunoassay with a suspected diagnosis of rheumatic disease by treating clinicians were taken for the study. The samples with a request for ANA by any method other than IIF, or accompanied by a request with a non-rheumatologic history are excluded. [7-8]

The serum samples which were positive for ANA by IIF method or those which were negative by IIF method but requested by the rheumatologists on clinical grounds were further processed for line immunoassay. Nylon strips coated with recombinant and purified antigens as discrete lines with plastic backing (EUROIMMUN AG) coated with antigens nRNP / Sm, Sm, SSA, Ro-52, SSB, Scl-70, PM-Scl, PCNA, Jo-1, CENP-B, dsDNA, nucleosomes, histones, ribosomal protein-P, anti-mitochondrial antibodies (AMA-M2) were used, along with a control band. [9] The nylon strip was incubated with serum at a 1:100 dilution. The test strips, thus, processed at a 1: 10 dilutions were analyzed by comparing the intensity of the reaction with positive control line by image analysis [Figure 1]. [10]

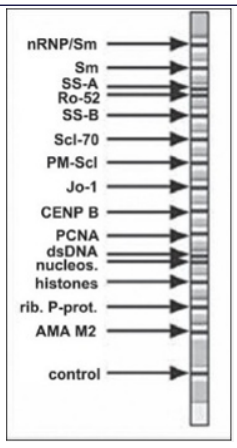

Figure 1 :Incubated EUROLINE ANA profile 3 showing the panel of auto anti bodies tested using EuroLineScan obtained from the kit literature provided by Euroimmune in authors laboratory.

Since we have done the statistical analyses only to the results obtained from the tests already done. So no ethics committee permission is required

RESULTS

In this prospective and retrospective study in a 12-month period,1024 samples were received. 1024 samples were analyzed for both ANA by IIF method and line immunoassay in this study. Among these 1024 samples, 404 (50.37%) were ANA-IIF positive in a 1:100 serum dilution. Of these positive ANA-IIF, 224 (21.75%) were also line immunoassay positive.

Total number of samples with ANA-IIF and line immunoassay done: 1024
Number of ANA-IIF positive with line immunoassay positive : 224
Number of ANA-IIF positive with line immunoassay negative : 180
Number of ANA-IIF negative with line immunoassay positive : 119
Number of ANA-IIF negative with line immunoassay negative :501

The various ANA patterns seen in the positive samples by IIFT Tests

Table 1. SPECTRUM OF ANA IIF PATTERNS OBSERVED IN THE STUDY POPULATION

ANA PATTERNS	SAMPLES(n=404)(%)	
Speckled(coarse& fine)	179	(44)
Speckled with cytoplasmic	38	(9.40)
Homogenous	50	(12.4)
Cytoplasmic	45	(11.13)
Nucleolar	44	(10.9)
Nucleolar dots	19	(4.7)
Centromere	9	(2.2)
Spindle shaped	8	(2.1)
Nucleolar homogenous	11	(2.72)
Golgi pattern	1	(.24)

The Speckled pattern was the most common ANA IIF pattern, seen in 179 (44.30%) of the positive404 samples. In comparison with line immunoassay results of these samples , various combinations of specific auto-antigens were observed and the most prevalent among these are shown in . The second most commonly occurring ANA pattern in this series was the Speckled with cytoplasmic (n = 38; 9.40%) pattern. The third ANA pattern Homogenous in nature (n=50,12.4%),Fourth most common pattern observed in our lab was only cytoplasmic pattern(n=44,10.9%),Nucleolar pattern was also prevalent in our department(n==44,10.9%), Another there are various types of ANA Patterns observed in our lab ,these are shown in Table 1.

TABLE 2 Distrubution of ANA HEP2 Patterns in different Age Groups(n=404)

Age distrubution	Speckled 179(44.30%)	Homo 50(12.37%)	Spec +cyto38(9.4%)	Cytoplasm 45(11-3%)	Nucleolar- 44(10.9%)	Centromere- 9(2.2%)	Spindle- 8(1.9%)	Nuclear dot- 20(4.8%)	N+ H- 11(2.7%)
0-10 year - F-12	7(3.9%)	(%)						5(25%)	
M-6	2(1.11%)			3(6.6%)				1(5%)	
11-20year -F -55	11(6.1%)	9(18%)	8(21%)	12(26.6%)	9(20.5%)			6(30%)	
M -9	1(.5%)	1(2%)		2(4.4%)		3(33.3%)	2(25%)		
21-30 year –F-81	43(24%)	13(26%)	4(10.5%)	5(13.15%)	7(15.5%)	2(22.2%)	3(57.5%)	3(15%)	1(.9%)
M-6	2(1.1%)	2(4%)	1(2.6%)			1(11.1 %)			
31-40year -F -107	77(43%)	12(24%)	6(15.7%)	3(6.6%)	6(13.6%)		1(12.5%)	2(20%)	
M-8	3(1.6%)	12(%)			3(6.6%)	1(11.1%)			
41-50year-F-59	22(12.29%)	6(12%)	14(36.8 %)	8(17.7%)	9(20.5%)				
M-7	2(1.11%)	12(%)	2(5.2%)			2(22.2%)			
51-60year-F-42	9(5%)	3(6%)		9(20%)	9(20.5%)		2(25%)	1(5%)	10 (90.9 %)
M-12	1(.5%)	2 (7.8%)	3(7.8%)	3(6.6 %)	1(2.2%)			2(%)	

Table 2 shows Distribution of ANA HEP2 Patterns in different Age Groups, Speckled patterns are common in 31-40 year age group. And females are more prevalent77/179(43%).Homogenous pattern more common in 21 to 30 age groups, females are more prevalent, 13/50(26%) ,Speckled with cytoplasm patterns are more common in 41-50 years. Female samples were shown positivity (14/38) (36.8%).Centromere, nucleolar patterns were shown different age group positivity. Nucleolar patterns are more common in 11-20 year age group,6/20(30%)Nucleolar homogenous patterns are more common in 51-60 group10/11(90.9%)

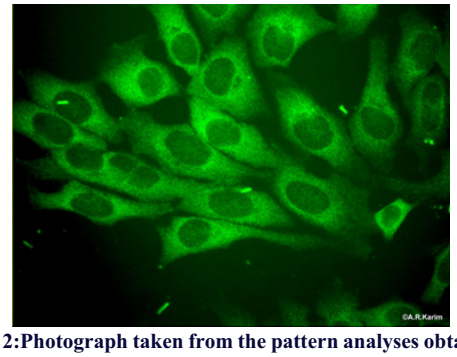

Figure 2:Photograph taken from the pattern analyses obtained in authors lab after doing IIFTS

RESULT AND DISCUSSION

First of all we must comment that both IIFT and Line Immunoassays should go hand on hand to explore the autoimmune spectrum of diseases as we can see from our study that the pictures coming from both IIF and Line immunoassays are quite varied, if one skipping the diagnosis but other still able to capture. We can also opine that we should do both the tests to tag one sample negative as there are a lot of cases where sample becomes positive after becoming negative from either IIFT or Line immunoassays.

We have done a detailed analysis of different patterns found in ANA Hep -2 analyses. 44% tested positive sample are found to be associated with speckled (Coarse and fine) pattern, similar finding has been reported by Zafer Mengeloglu et al in their study, but what we have done we tested the pattern further to see cytoplasmic part and have found that it is 9.4% which shows cytoplasmic granularity as well, thus makes it more precise to diagnose systemic sclerosis.

It was also found that in line immunoassay, most common finding was SSA/Ro-52 positivity(34.17%) thus favoring the diagnosis of systemic sclerosis .We must reiterate that in our geographical area patients are suffering more with systemic sclerosis from the wide basket of auto immune diseases.

Now taking a good look towards other patterns, it was thoroughly studied to find that the second most common ANA IIF pattern is

Homogenous pattern. Among them 41(82.04%) samples is positive for line immuneassay.9 (18%) samples are line immunoassay negative. Various combination of specific nuclear auto antigens were observed .Most common pattern DsDNA, Nucleosome, SSA/RO-52(26.82%).Here also the diagnosis tilted between SLE and Systemic Sclerosis.

Immunoassay of 156 samples with ANA-IIF centromere, nucleolar and Nuclear Dots, Cytoplasm, Speckled& cytoplasm patterns reveal that Centromere patterns were observe in 9 samples (2.2%),7 samples shows positivity for line immunoassay(77.7%).samples shows positivity for CENP-B(57.14%)& specific combination of antigens RNP/Sm/SSA/RO-52(42.9%).This finding was consistent with CREST syndrome clinical and ANA picture point of view.

From the age group and sex distribution analysis we can say that it is the female preponderance among all age group though that was quite expected but what is interesting to note that among male the age group modal occurrence occurs around age group of 31-40.So from the clinical diagnosis point of view catch them young should be our main motto.

CONCLUSIONS

So in nutshell what we can opine that there are a lot varied result coming out of our study to necessitate a hand to hand examination of both ANA Hep-2 and Line immunoassays to help clinicians to reach the diagnosis what was thought previously a pure clinical entity.

The Pattern analysis clearly favoring a diagnosis of systemic sclerosis that too occurring an age group of 31-40 preponderance in male and equally distributed in all age groups among female in this geographical area. So if we can with the help of these wonderful cost friendly easy tests diagnose the sufferers at early stages of diseases we can thus help clinicians to make symptom free and healthy life span for this dreaded disease bearers.

Conflict of Interest: No Such

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