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THE PATTERN RECOGNITION OF AUTO IMMUNE CONNECTIVE TISSUE DISORDER WITH THE HELP OF ANTINUCLEAR ANTIBODY DETECTION BY INDIRECT IMMUNOFLUORESCENT MICROSCOPY AND LINE IMMUNOASSAY IN THE REGION OF WEST BENGAL :A CROSS SECTIONAL STUDY

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ABSTRACT

Introduction: Auto immune Connective Tissue Disorder (CTD) is a multisystem Disorder with pathogenesis depending upon the different proteins and cells of the body acting as foreign antigen or on other hand our immune system is becoming overactive to recognize host protein or cells as foreign. To treat this group of patients we need to have a diagnostic system which can have the power to detect the disease early and able to differentiate between different types of the Auto Immune CTD so that patients can be treated according to the diagnosis and not just by empirical generalized treatment.

Objectives: 1) The Pattern analysis among different sub groups of CTD in the disease population2) The Pattern analysis among patients screened positive by ANA Hep2 method in the disease population3) To estimate the Prevalence of Different groups of CTD arising out of pattern analysis.

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)

Result and Discussion: We have found that among total cases tested positive it was a staggering 44% found to be speckled type. We have studied a little deeper, what we have done we took the positive sample as Screened by ANA HEP 2 to run for ANA Profile IMMUNOASSAY technique. We have found that the Speckled type was only positive in line IMMUNOASSAY technique by a mere 65%.Different pattern of Antibody Combination was also found in line IMMUNOASSAY chiefly revolved round SSA and SSB type.

In the Second Common general pattern as shown by ANA HEP 2 was Homogeneous pattern Which on further analysis showed a line IMMUNOASSAY positivity rate of 82% and chief antibody found was Anti Ds DNA and RO-52 combination.

Summary and Conclusion: We thus conclude that our study tool with Hep2 and ANA Profile Screening should be done HAND TO HAND to delineate the Auto Immune CTD scenario in our part of world and design a combat force against this spectrum of dreaded disease.

KEYWORDS : ANA Hep2,ANA profile, CTD,SSA,SSB,Anti DsDNA

INTRODUCTION

Auto immune Connective Tissue Disorder (CTD) is a multisystem Disorder with pathogenesis depending upon the different proteins and cells of the body acting as foreign antigen or on other hand our immune system is becoming overactive to recognize host protein or cells as foreign.

To treat this group of patients we need to have a diagnostic system which can have the power to detect the disease early and able to differentiate between different types of the Auto Immune CTD so that patients can be treated according to the diagnosis and not just by empirical generalized treatment.

The most important trademark of Auto immune CTD is the presence of Anti-Nuclear Antibody in the cell. There is a lot of diagnostic methods are available to detect this but to do a screening as well as diagnosis at the same time by a single test followed by quantification ,the usage of Indirect Immuno Fluorescence Microscopy With HEP 2 method with Strip Immuno Assay with ANA Profile Analysis holds the key.

Due to high sensitivity & Specificity, Indirect Immunfluorecence (IIFT) using (Human epithelial cells & primate liver) is thought to be 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]

The patterns of the test results showing a gross nature of disease in different parts of India as well as globally but there is existing gap in the scenario regarding the pattern of distribution in age groups and sex groups in our part of world .We know that the age group distribution as well sex distribution hold key when we are treating the patients as every group has its own characteristics when it comes to the pathogenesis of any disease.(4)

To bridge the gap in the existing knowledge we wanted to study the different patterns of The IIFT pictures with reference to certain specific age groups and sex groups in the blood samples sent to Biochemistry Departments after proper Consent taken from the patients while coming for treatment in the Rheumatology OPD of the Tertiary Care Hospital Catering a large population of the state West Bengal.

OBJECTIVES

1) The Pattern analysis among different sub groups of CTD in the disease population
2) The Pattern analysis among patients screened positive by ANA Hep2 method in the disease population
3) To estimate the Prevalence of Different groups of CTD arising out of pattern analysis.

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)

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. [5]

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. [6] 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]. [6]

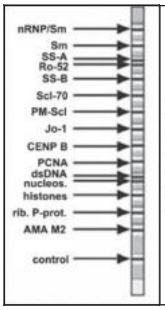

Figure 1 :Incubated EUROLINE ANA profile 3 showing the panel of auto anti bodies tested using EuroLineScan obtained from the kit literature provided by Euroimmune in authors laboratory.

RESULTS

Table 1 Immuneassay Details Of 179 Samples With ANA -IIF Speckled Pattern(coarse And Speckled);

IMMUNEASSAY POSITIVE: 117(65.36%)
IMMUNEASSAY NEGETIVE: 62(34.63%)

Line immuneassay specificity	(n =117)Immuneassay positive(%)
SSA/RO -52/SSB	47 (34.17)
RNP/Sm/SSA	41 (33.04)
SSA/RIB	11 (9.4)
RNP/Sm,SSA/RO-52.SSB,	13 (11.1)
Scl 70	5 (4.2)

Correlation with line immunoassay results shown in Table 1.Here Speckled pattern observed by ANA IIF method (n=179),but among them immunoassay positive 117 samples (65.36%),Immunoassay negative 62(34.63%).In Immunoassay technique various combination of specific auto antigens were observed and the most prevalent among these are shown in table 1,here SSA/RO-52/SSB antigens combination were found positive(34.17%).RNP/Sm/SSA nuclear auto antigens also found positive(33.04%).

Table 2:Immunoassay Details Of 50 Samples With ANA-IIF Homogenous Pattern

Line immunoassay **positive -41(82%)** samples **Negative-9** (18%) samples

LINE IMMUNASSAY	Samples (**n-41)(%)**
DsDNA,Nucleosome,Histone	9(21.95%)
DsDNA,Nucleosome, SSA/RO-52	11(26.82%)
DsDNA,Nucleosome, RIB	9(21.95%)
DsDNA.RNP/SM	7(17.07%)
RIB/SSA	1(2.4%)
SCL 70	4(9.7%)

Table 2 is showing that 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 immuneassay negative.various combination of specific nuclear autoantigens were observed .Most common pattern DsDNA,Nucleosome,SSA/RO-52(26.82%) & another patterns are shown in Table 3.

Table 3: Immunoassay details of 156 samples with ANA-IIF centromere, nucleolar and Nuclear Dots, Cytoplasm, Speckled& cytoplasm patterns

Pattern(ANA IIF method) (n=404)	Samples (positive Line immunoassay)	Line immunoassay Specificity
Centromere (total sample-9)(2.2%)	7(77.7%)	CENP-B -4(57.14%) RNP/sm/SSA/RO52-3(42.9%)
Nucleolar(total sample-44)	12(27.27%)	PCNA-7(58.3%) SCL70-3(25%) SSB-1(7.6%) RNP/SSA-1((7.6%)
Nuclear Dots (total sample-19)	4(20%)	Ssa-2(50%) Ro52-2(50%)
Cytoplasm(total sample-45)	15(33.33)	PCNA-2(13.3%) DsDNA-3(20%)AMA-M2-3(20%)RIBP-7(46.6%)
Speckled& cytoplasm(total sample-38)	13(34.21%)	Ssa/rnp/sm/ro52-12(92.3%) Rib-1(7.6%)

Table 3 shows the different patterns observed among 156 samples but line immuneassay positive .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 antigensRNP/Sm/SSA/RO-52(42.9%).Details were shown in Table 4

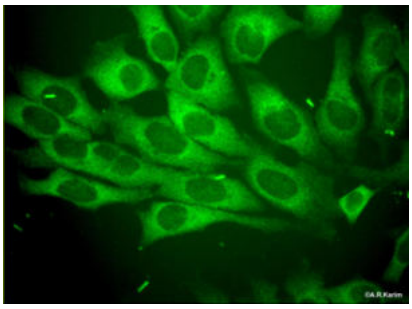

Figure 2:Photograph taken from the pattern analyses obtained in authors lab after doing IIFTS

Table 3 Immuneassay Details Of 119 Samples With Immuneassay Positive:119 But ANA HEP2(IIFT) NEGATIVE

Line immuneassay specificity	(n =119)Immuneassay positive (%)
SSA/RO -52/SSB	36
RNP/Sm/SSA	31
DsDNA	35
PCNA	3
Scl 70	6
Anti centromere antibody	5
Jo1 antibody	3

RESULT AND DISCUSSION

We have found that among total cases tested positive it was a staggering 44% found to be speckled type. We have studied a little deeper, what we have done we took the positive sample as Screened by ANA HEP 2 to run for ANA Profile IMMUNOASSAY technique. We have found that the Speckled type was only positive in line IMMUNOASSAY technique by a mere 65%.Different pattern of Antibody Combination was also found in line IMMUNOASSAY chiefly revolved round SSA and SSB type.

In the Second Common general pattern as shown by ANA HEP 2 was Homogeneous pattern Which on further analysis showed a line IMMUNOASSAY positivity rate of 82% and chief antibody found was Anti Ds DNA and RO-52 combination.

In the next step we have done the detail analysis of other patterns found in ANA HEP 2 method. We found that there were different combinations of Antibody found positive with line IMMUNOASSAY. Another aspect of the testing of ANA profile is also reflected in the fact that though Ana Hep 2 screening seems to be the diagnostic test but if we can go a bit further to reexamine the samples with ANA profile strip ELISA method it would do a world of good to catch the patients affected with any kind of Auto immune CTD.

We have found that some of the Positive ANA Hep2 patients showing Negative ANA profile test results and also the opposite.

SUMMARY AND CONCLUSION

In Different Previous studies and unpublished works we have found that Autoimmune CTD has been categorized on the basis of its pattern in auto nuclear antibody screening procedure. It was also found that in our part of the world that it was the speckled pattern which held the first position in the pattern analysis. We have gone a little further. We have not only characterizes the gross pattern in Hep 2 but also checked the subset of the findings in each gross pattern like Speckled pattern,

Homogeneous pattern, Cytoplasmic pattern, Nucleolar pattern etc. We have come to know that it was the SSA and SSB type which showed the maximum frequency among cases of speckled pattern.Ro-52 and Anti Ds DNA combination showed fair distribution among patients with Homogeneous Hep2 characteristics.

We thus conclude that our study tool with Hep2 and ANA Profile Screening should be done HAND TO HAND to delineate the Auto Immune CTD scenario in our part of world and design a combat force against this spectrum of dreaded disease.

Conflict of Interest: Nil

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