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DIAGNOSTIC FINDING OF VARIOUS FLUORESCENCE PATTERN IN IIFT INDICATING PRESENCE OF ANA IN CONNECTIVE TISSUE DISEASED PATIENTS OF SUB- HIMALAYAN POPULATION

Biochemistry

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ABSTRACT

Background and objective: For the management of connective tissue disorders (CTDs), antinuclear antibody (ANA) testing is essential. Indirect immunofluorescence (IIF) using human epithelial cells and primate liver is considered the gold standard for the screening of ANA. In this ethnic population of North Bengal where CTDs are common, if ANA-IFA pattern's correlation is reliably known ,it can minimize the cost of managing CTDs by costlier monospecific methods. **Method:** Serum samples of 1763 suspected CTD patients attending OPD of North Bengal medical college were tested for ANA by IIFT . **Result** : Different ANA-IIF patterns were found on microscopy like fine speckled, coarse speckled, homogenous, centromere, nucleoli, nucleoplasm, nuclear membrane , spindle apparatus ,nuclear dots etc. These likely to be associated with specific antigens can be further differentiated by immuoblot or ELISA . **Conclusion:** We found different typical fluorescence signal patterns of the samples associated with specific autoantibodies which would help in the diagnosis of various CTD by further differentiation.

KEYWORDS

ANA, IIFT, CTD

INTRODUCTION

Connective tissue diseases (CTDs) like Systemic Lupus Erythematosus (SLE), Rheumatoid arthritis (RA), Sjogren's syndrome (SS), Systemic sclerosis (SSc), Polymyositis (PM), Mixed connective tissue disease (MCTD), and so on. elicit a generalized autoimmune response with generation of Antinuclear Antibodies (ANAs)[1]. Anti-nuclear antibodies (ANA) represent important diagnostic markers in these autoimmune rheumatic conditions[2-6]. Low-titer ANA may also be detected in healthy individuals[7,8]. ANA is commonly used to encompass not only antibodies directed against nuclear antigens, but also those binding to constituents of the nuclear envelope, mitotic spindle apparatus, or cytoplasm.[6]

In 1957, the first ANA was demonstrated by indirect immunofluorescence (IIF) in the serum of SLE patients, followed by the discovery and characterization of extractable nuclear antigens in 1959 [9,10]. IIF testing has since become the standard method for ANA screening in patient sera, using human epithelial cells (HEp-2) . Hep-2 cells present a very broad spectrum of 100–150 cell antigens at different stages of the cell cycle, allowing the sensitive detection of numerous clinically relevant autoantibodies[6]. Though for high demand of ANA testing,there has been a movement from IIF to largely automated screening methods like ELISA and flow cytometric bead-based ("multiplex") immunoassays that are based on a limited number of purified and/or recombinant antigenic substrates, Samples classified as positive through screening by ELISA or multiplex are usually reflexed to IIF to confirm the result and to determine the titer and associated ANA pattern(s), while samples devoid of reactivity against the antigenic panel are reported as negative.[6]

ANA IIF screening provides information on antibody patterns and titers, followed by a confirmatory monospecific test (e.g., ELISA, Multiplex, and immunoblot) to identify the autoantibody[11]

MATERIALS AND METHOD:

The present study is an institutional based observational comparative study conducted among suspected CTD patients (0-60) years irrespective of gender attending North Bengal Medical College and hospital , a tertiary care centre in the sub Himalayan belt. Serum samples of 1103 suspected CTD patients from rheumatology Dept., pediatrics and dermatology dept. and Medicine dept. were sent for ANA-IIF testing to the Biochemistry dept. ANA-IIF was performed using Euroimmune Mosaic HEp2 and Primate liver cell (PLC) substrate (Euroimmune AG, Germany, Lübeck) as per kit insert instruction. Serum was diluted in a ratio of 1:100 and slides were examined under a fluorescent microscope at 40X magnifications.
The fluorescence intensity was scored semi-quantitatively from 1+ to 4+ relative to the positive control (4+). Results for ANA patterns and calculated titers were entered into laboratory information system and statistical software SPSS (version 22 for analysis.

RESULTS:

During the study period, we received 1013 serum samples from mid February 2022 to mid June 2023 for ANA testing. Out of it, 800 cases were found positive in IIFT showing typical fluorescence pattern while 213 were found negative with no pattern. Throughout this study, the biochip slides coated with HEp-20-10 cells were of consistent quality. Sex based distribution showed that only 65 among the positive cases were males while the rest of them (735) comprised of females and that too mainly between (24-45)yrs age group

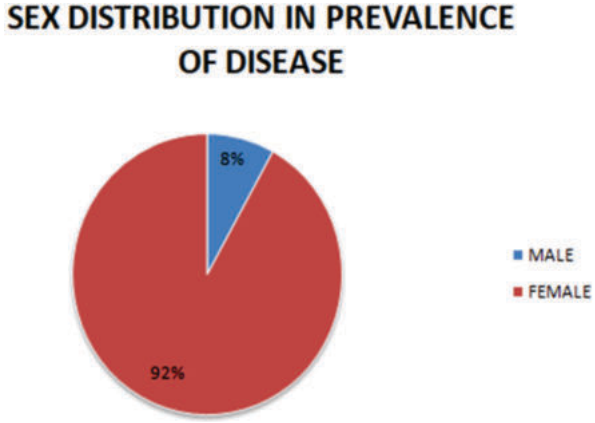
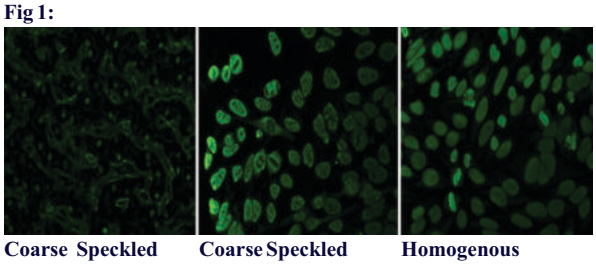
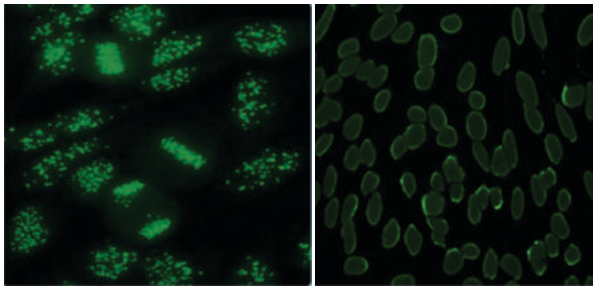


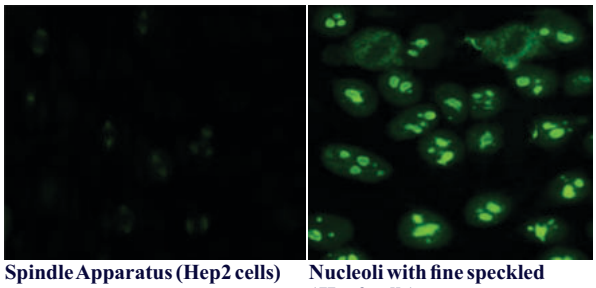
Fig 1:



Coarse Speckled (primate) | **Coarse Speckled (Hep2 cells)** | **Homogenous (Hep2 cells)**



Centromere (Hep2 cells) Nuclear Membrane (Hep2 cells)



Spindle Apparatus (Hep2 cells) Nucleoli with fine speckled (Hep2 cells)

Fig 2: Principal ANA-IIF patterns observed in the current study

Table 1: Evaluation Of Different Ana Patterns

Specimen Patterns	Number (n)	Percentage %	Known Target Antigen	Clinical Association
Fine speckled	278	34.75	mi-2,Ku,SSA,SSB,TIF1 gamma,PCNA,Rib,SRP	SLE,PM/DM
Coarse speckled	149	18.62	HnRNP,U1Nrnp,Sm,Rna Pol111	SLE, MCTD
Mixed pattern	120	15	NuMA(MSA-1)	SJS,APS,SLE
Homo genous	79	9.80	dsDNA, ssDNA, nucleosomes, histones, Scl70	SLE,DLE JIA,PSS
Nucleoli	41	5.12	PM-Scl, U3-Nrnp/fibrillarin,rnaPol 1	PM, PSS
Spindle pole	36	4.50		
Cytoplasm	27	3.37	Vimentin, tropomysin, Rib, Jo1	SLE,RS,PM
centromere	18	2.25	CENP-A,CENP-B	PSS
Spindle apparatus	14	1.75	HsEG5	SJS,SLE
Nucleoplasm	13	1.62	SSA,SSB	SLE, SJS
Midbody	8	1		RS,PSS
DFS	5	0.62	DFS70	AD, Asthma
Multinuclear dots	4	0.5	Sp100,Sp140,PML	PBC,RD
Nuclear membrane	4	0.5	Gp210,laminA,lamin B	PBC
PCNA like pattern	3	0.37	PCNA	SLE
Few nuclear dots	1	0.12	P80-collin,SMN,Nor90	SLE,SJS, PSS
Total positive	800	100		
Negative	213			
Total samples	1013			

- SLE- systemic lupus erythromatosus, SJS- Sjogren's syndrome, PM- Polymyosites
- DM-dermatomyosites, PSS- progressive systemic sclerosis, AD-atopic dermatitis
- PBC- Primary billiary cholangites, RS- Raynaud's syndrome,
- JIA- juvenile idiopathic arthritis, RD- Rheumatic diseases
- MCTD- Mixed connective tissue disorder

DISCUSSION:

Joint involvement is common in CTD patients. Autoimmune diseases are associated with autoantibodies of which some are pathogenic[12] however; screening for specific autoantibodies in patients is not routinely performed. Our study showed that females of middle age groups were mainly affected. Diagnostic performance for ANA patterns by IIFT was evaluated using 800 positive samples. Prevalence of ANA patterns in this sub- Himalayan population of North Bengal showed fine speckled fluorescence of nucleus the most followed by coarse speckled, mixed pattern, homogenous, nucleoli,spindle pole, centromere, midbody etc along with some even cytoplasm patterns noted though non-specific. This indicated presence of autoantibodies in serum against probable target antigens. The target antigens can be futher confirmed by immunoblot technique for ANA or myosites or sclerosis profile thereby reflecting the diagnosis of the probable specific disease. . However, improvements in the pattern and titer recognition and clinical specificities are necessary.

CONCLUSION:

Screening for specific autoantibodies by IIFT and their proper pattern recognition can help in assessment as well as prevention of various connective tissue disorders.

REFERENCES:

1. Kalita, D., Rekha, U. S., Raj, A. K., Mahanta, P., Gupta, P., & Deka, S. (2022). Feasibility of Indirect immunofluorescence (IIF) alone as a screening method for antinuclear antibody in connective diseases in India's sub-Himalayan region. *Indian Journal of Pathology and Microbiology*, *65*(4), 873.
2. Bizzaro, N. (2007). Autoantibodies as predictors of disease: the clinical and experimental evidence. *Autoimmunity reviews*, *6*(6), 325-333.
3. Damoiseaux, J., Andrade, L. E., Fritzler, M. J., & Shoenfeld, Y. (2015). Autoantibodies 2015: from diagnostic biomarkers toward prediction, prognosis and prevention. *Autoimmunity reviews*, *14*(6), 555-563.
4. Arbuckle, M. R., McClain, M. T., Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., & Harley, J. B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *New England Journal of Medicine*, *349*(16), 1526-1533.
5. Wiik, A. S. (2005). Anti nuclear autoantibodies: clinical utility for diagnosis, prognosis, monitoring, and planning of treatment strategy in systemic immunoinflammatory diseases. *Scandinavian journal of rheumatology*, *34*(4), 260-268.
6. Ricchiuti, V., Adams, J., Hardy, D. J., Katayev, A., & Fleming, J. K. (2018). Automated processing and evaluation of anti-nuclear antibody indirect immunofluorescence testing. *Frontiers in Immunology*, *9*, 927.
7. Tan, E. M., Feltkamp, T. E. W., Smolen, J. S., Butcher, B., Dawkins, R., Fritzler, M. J., ... & Koziol, J. A. (1997). Range of antinuclear antibodies in "healthy" individuals. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, *40*(9), 1601-1611.
8. Vrethem, M., Skogh, T., Berlin, G., & Ernerudh, J. (1992). Autoantibodies versus clinical symptoms in blood donors. *The Journal of rheumatology*, *19*(12), 1919-1921.
9. Friou, G. J., Finch, S. C., Detre, K. D., & Santarsiero, C. (1958). Interaction of nuclei and globulin from lupus erythematosis serum demonstrated with fluorescent antibody. *The Journal of Immunology*, *80*(4), 324-329.
10. Holborow, E. J., Weir, D. M., & Johnson, G. D. (1957). A serum factor in lupus erythematosus with affinity for tissue nuclei. *British medical journal*, *2*(5047), 732.
11. Tozzoli, R., Bizzaro, N., Tonutti, E., Villalta, D., Bassetti, D., Manoni, F., ... & Rizzotti, P. (2002). Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. *American Journal of Clinical Pathology*, *117*(2), 316-324.
12. Selvaraja, M., Daud, C. K. D. C. K., Jalil, M. A., Shah, A. M., Amin, S., Nordin, Z. A. B., ... & Abdullah, M. (2018). Indirect Immunofluorescence Screening of Potential Arthritic Autoantibodies in Systemic Lupus Erythematosus. *Malaysian Journal of Medicine and Health Sciences*, *14*(103).