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A STUDY OF AUTOIMMUNE CONNECTIVE TISSUE DISEASES IN A TEACHING HOSPITAL IN SOUTH KARNATAKA

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ABSTRACT **Introduction:** The autoimmune connective tissue disorders (ACTD) have since long been reported from the Western world. Non availability or availability of incomplete epidemiological data and lack of advanced diagnostic facilities in developing countries has probably been the reason for under diagnosis in many cases. Objective of the study to determine the autoimmune connective tissue diseases of suspected cases by ANA IIFT and line immunoassay. **Method:** In this study, we performed a retrospective analysis of medical records of all clinically suspected autoimmune connective tissue disease cases whose sera were sent for antinuclear antibody testing. The ANA-IIF positive cases were further analyzed to find out the specific profile of antibodies by Immunoblot assay. **Result:** A total of 480 serum samples were screened for antinuclear antibody (ANA) from April 2015 to April 2016. Out of these 107 were positive by a screening test, and 94 by Immunoblot test. A female predominance was also noted with a female-to-male ratio of 3:1. ANA-IIF test screen was positive in 26.5% of clinically suspected patients. Among the ANA-IIF positive samples, 94 were positive by the specific immunoblot test. The most common ANAs found were anti ds-DNA antibodies followed by anti-U1-RNP antibodies. **Conclusion:** Though, SLE is the major autoimmune connective tissue disorder in our local population, MCTD and Sjogren's syndromes are also frequent.

KEYWORDS : Autoimmune, Antinuclear Antibodies, Immunoblot assay.

INTRODUCTION:

A systemic autoimmune response is a common manifestation of the rheumatic connective tissue disease (CTD) and its hallmark is the presence of antinuclear antibodies (ANA). Indirect immunofluorescence (IIF) on HEp-2 (human epithelial cell tumor line) is the classical technique used for the detection of ANA, which is also used as a screening test [1]. The autoimmune connective tissue disorders (ACTD) have since long been reported from the Western world. The detection of autoantibodies against the cell nuclei (antinuclear antibodies) and cytoplasmic components plays an important role in the diagnosis of many autoimmune diseases such as Systemic lupus erythematosus (SLE), Mixed connective tissue disease (MCTD), Rheumatoid arthritis, Sjogren's syndrome, Progressive systemic sclerosis etc. Accurate diagnosis of the autoimmune disease is important for disease monitoring, and prognostic assessment.

The autoimmune disorders (AID) represent a global health problem. Worldwide, they are responsible for considerable morbidity and mortality and come next only to cancer and atherosclerosis. In Western countries, they are known to affect 3–5% of the population [2]. The incidence in the past few years seems to be on the rise not only in Western but also in developing countries [3].

Aim of the objective is to determine the autoimmune connective tissue diseases of suspected cases by ANA IIFT and line immunoassay.

MATERIAL AND METHODS:

In this study, we performed a retrospective analysis of medical records of all clinically suspected autoimmune connective tissue disease cases whose sera were sent for antinuclear antibody testing. The ANA-IIF positive cases were further analyzed to find out the specific profile of antibodies by Immunoblot assay.

Serum samples of patients who sought medical help for 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 12 months.

Fresh whole blood samples were collected with patient consent at the phlebotomy section. Serum separated from the clotted blood samples by centrifugation. A fasting blood sample was most often used to avoid lipemic serum which could result in increased background fluorescence or unclear staining pattern [1].

All serum samples, thus received with a request for the ANA by IIF method and line immunoassay with a suspected diagnosis of rheumatic disease by treating clinicians were taken for the study.

Serum samples were processed in a dilution of 1:100 using HEp–2010 / liver biochip (Monkey) (EUROIMMUN AG) and conjugated with specific antihuman IgG (EUROIMMUN AG) [4]. The fluorescence intensity was scored at x 400, semiquantitatively from 1+ to 4+ relative to the intensity of the positive (4+) and negative control [5]. The test result was discarded if the positive control sample failed to show the precise results. The serum samples which were positive for ANA by IIF method or those which were negative by the 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. 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.

RESULT:

A total of 480 serum samples were screened for antinuclear antibody (ANA) from April 2015 to April 2016 in Yenepoya Medical College & Hospital Mangalore, Karnataka. Among the connective tissue disorders (CTD): Systemic Lupus Erythematosus (SLE) was the most common clinical diagnosis (154/480 cases) followed by Mixed Connective Tissue Disease (MCTD) (136/480 cases), Sjogren's syndrome (113/480 cases), CREST/ Scleroderma (60/480 cases) and Myositis (17/480 cases) as shown in figure 1. Out of 480 serum samples, 107 (22.3%) were positive by the ANA-IIF test in a 1:100 serum dilution, as shown in table 1. A female predominance was also noted with a female to male ratio of 3:1. ANA-IIF test screen was positive in 22.3% of clinically suspected patients. Of these ANA-IIF positive samples, 94 (87.9%) were positive by the specific immunoblot assay. The most common ANAs found 32/94 were anti ds-DNA antibodies followed by 24/94 anti-U1-RNP antibodies, 20/94 were SSA/Ro-52, SSB antibodies, 12/94 were CENB-B, Scl-70, 05/94 were Jo-1, 01/94 were AMA–M_2-1, as shown in table 2. Among these positive connective tissue diseases, 34.1% were SLE, 26.6% MCTD, 21.3% were Sjogren's syndrome, 2.7% were CREST/Scleroderma, and 5.3% were Myositis, as shown in table 3.

Table 1: Spectrum Of ANA IIF Patterns Of 107 Samples

ANA IIF Patterns (n=107)	Sample n
Nucleolar + Homogenous	16
Speckled	02

Coarse granular	20
Nucleus + coarse granular	53
Cytoplasm + filamentous	02
Mixed	14
Total	107

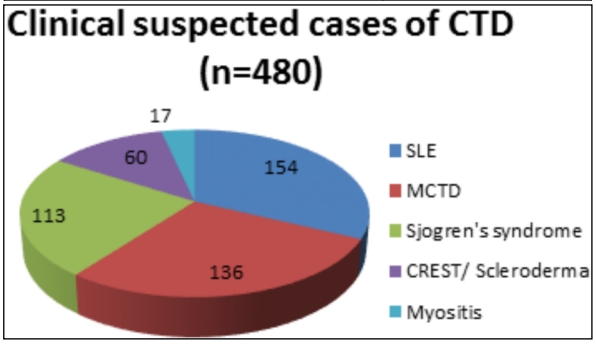

Figure 1: Clinical Suspected Cases Of CTD

Table 2: Line Immunoassay Details Of 94 Samples

Line immunoassay specificity (n = 94)	Samples n (%)
dsDNA, Nuclosome, Histone, rib P protein	32 (34.1)
U1- SnRNP	24 (25.5)
SSA/Ro-52, SSB	20 (21.3)
CENB-B, Scl-70	12 (12.7)
Jo-1	5 (5.3)
AMA –M_2-1	1 (1.1)

Table 3: CTD Identified By Immunoblot Test:

CTD (n=94)	Positive test n (%)
SLE	32 (34.1%)
MCTD	25 (26.6%)
Sjogren's syndrome	20 (21.3%)
CREST/ Scleroderma	12 (12.7%)
Myositis	05 (5.3%)

DISCUSSION:

The clinical manifestations in connective tissue diseases vary, ranging from a typical malar rash to the atypical involvement of the CNS. The physicians depend largely on clinical features to diagnose, but in a large majority of undifferentiated cases, a laboratory test is required to prove or disprove the possibility of autoimmune etiology. In a present study 107/480 (22.3%) were ANA-IIF positive in a 1:100 serum dilution, of which 94/107 (87.9%) were line immunoblot assay positive. A study done by Sebastian W *et al*., (2010), analysed 319 samples of suspected connective tissue disease, and identified that 38.2% (122/319) were ANA-IIF positive. Of these ANA-IIF positive samples, 101/122 (82.8%) were also line immunoassay positive for serum autoantibodies. In these 101 positive samples, the homogenous pattern was the most common ANA pattern (45.5%), followed by the speckled pattern (35.6%), which is not inaccordance to the present study [1]. The present study had showed the most common ANA pattern was nucleus, coarse granular pattern followed by homohgenous pattern. A study conducted by Kosaraju K *et al*., (2010), on 48 South Indian patients with a clinical diagnosis of SLE, ANA was positive only in 64.28% and anti-dsDNA was positive in 89.36% of patients. Homogenous pattern of immunofluorescence was the most common. ANA profile showed positive reactions with dsDNA, RNP and Sm antigens [6]. In another study done by Gilburd B *et al*., (2004), on patients with Sjögren's syndrome, none of the sera of 37 patients had autoantibodies reacting with Sm, Jo-1, dsDNA or histones. Anti-RNP antibody was found in 5.4% of the sera and 2.7% of the sera reacted with Scl-70. Anti-SS/A and anti-SS/B were identified in 84% and 76% of the sera respectively, which is high from the present study [7].

A study conducted by Behmanesh F *et al*., (2010), on 118 samples of systemic sclerosis patients showed that 94% of patients were ANA positive. The predominant patterns on HEp-2 cell substrate were speckled (70%), nucleolar (37%), and homogenous (19.4%). Maximum number of patients were positive for 30% Scl-70 autoantibody [8], Whereas present study had showed dsDNA nucleosome and histones was maximum i.e., 34.1%. In our study population, SLE is the major autoimmune connective tissue disorder closely followed by MCTD and Sjogren's syndromes. The positivity of ANA-IIF screen test varies from 20% to 93% and our result was consistent. In present study, 22.3% were ANA positive, which is inaccordance to a similar study by Minz RW, *et al*., (2012), reported 18.9 % were ANA positive [9].

CONCLUSION:

SLE is the major autoimmune connective tissue disorder in our local population, followed by MCTD and Sjogren's syndromes. Though detection of ANA is an important screening test for identification of antibodies against dsDNA, RNP/Sm and anti U1-RNP etc. has more reliable diagnostic and prognostic implications. Knowing to the chronicity and the ability to cause serious complications, early detection, diagnoses and management might go a long way in delaying serious systemic involvement. However, studies on clinical features and autoantibody profiles involving a larger population are required to validate the findings of the present study.

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Conflicts Of Interest:
No conflicts of interest.

REFERENCES:

1. Sebastian W, Roy A, Kini U, Mullick S. Correlation of Antinuclear Antibody Immunofluorescence Patterns with Immune Profile using Line Immunoassay in the Indian Scenario. Indian Journal of Pathology and Microbiology. 2010; 53:427-32.
2. Cooper GS and Stroehla BC. The epidemiology of autoimmune diseases. Autoimmun Rev. 2003; 2:119-125
3. Malviya AN, Singh RR, Singh YN, Kapoor SK, Kumar A. Prevalence of systemic lupus erythmatosus in India. Lupus. 1993; 2:115-118.
4. (EUROIMMUN AG)- Instructions for the Indirect Immunofluorescence test: Mosaic HEp–20-10/Liver (Monkey) version. EUROIMMUN AG, Germany. Available from: http://www. euroimmun.com.
5. Kavanaugh A, Tomar R, Reveille J, Solomon DH, and Homburger HA. Guidelines for Clinical Use of the Antinuclear Antibody Test and Tests for Specific Autoantibodies to Nuclear Antigens. Arch Pathology Lab Med. 2000; 124:71-81.
6. Kosaraju K, Shenoy S, Suchitra U. A Cross-sectional Hospital-Based Study of Autoantibody Profile and Clinical Manifestations of Systemic Lupus Erythematosus in South Indian Patients. Indian J Med Microbiol 2010; 28:245-247.
7. Gilburd B, Abu-Shakra M, Shoenfeld Y, Giordano A, Bocci BE, Monache FD *et al*. Autoantibodies profile in the Sera of Patients with Sjogren's Syndrome: The ANA Evaluation – A Homogenous, Multiplexed System. Clin Dev Immunol 2004; 11:53-56.
8. Behmanesh F, Amin R, Khajedaluee M, Fritzler MJ. Autoantibody Profile in Systemic Sclerosis. Acta Medica Iranica. 2010; 48:12-20.
9. Minz RW, Kumar Y, Anand S, Singh S, Bamberi P, Verma S *et al*., Antinuclear antibody positive -autoimmune disorders in North India: an appraisal. Rheumatol Int. 2012; 32:2883-2888.