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Diagnostic value of laboratory techniques in diagnosing Drug-Induced Lupus erythematosus among long-standing tuberculosis patients on treatment with Isoniazid

Sangita Biswas[1], Suparna Datta[1], Soumika Biswas[1], Subinay Datta[2]*, Harsh Nawal[3], Subhramay Chatterjee[4]

[1]Asssitant Professor, Department of Biochemistry, 88, College Street, Medical College, Kolkata-700073, West Bengal, India
[2]Associate Professor, Department of Biochemistry, 88, College Street, Medical College, Kolkata-700073, West Bengal, India
[3]Junior resident, Department of Biochemistry, 88, College Street, Medical College, Kolkata-700073, West Bengal, India
[4]Professor & Head, Department of Biochemistry, 88, College Street, Medical College, Kolkata-700073, West Bengal, India

Corresponding Author

Subinay Datta, Associate Professor, Department of Biochemistry, 88, College Street, Medical College, Kolkata-700073, West Bengal, India

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ABSTRACT

Background: Drug-induced lupus erythematosus (DILE) is a variant of lupus erythematosus that resolves within days to months after withdrawal of the culprit drug in a patient with no underlying immune system dysfunction. The etiology of these presentations presents as a diagnostic dilemma to the physician. Moreover, no existing literature could be found that studied the comparative antibody profiles of patientsdeveloping rash due to DILE and those developing rash due to other aetiologies. So, the differentiation was done based on the antibody profiles that are essential to help physicians. **Methods:** By stratified random sampling, the 11 study population was introduced into the study that had been diagnosed with having DLE and rash persists even after discontinuation of ATT. Thereafter, all of the patients persons were subjected to the estimation of serum ANA, anti-histone antibody, anti-dsDNA antibody and anti-ssDNA antibody, and find out C3 and C4 concentration. **Results:** it was found that among 95% patients have positive anti-histone antibody and near about 90% have antinuclear antibodies. Almost all patients were positive for anti-ssDNA. But anti-dsDNA was rare and serum concentration of C3 and C4 were normal. **Conclusion:** Serological profile of INH induced DIL in the study, it was found that among 95% patients have positive anti-histone antibody and 90% have antinuclear antibodies. Almost all patients were positive for anti-ssDNA. But anti-dsDNA was rare and serum concentration of C3 and C4 were normal.

KEYWORDS: Drug-Induced Lupus erythematosus, Tuberculosis, Isoniazid, Anti histone antibody, Anti-nuclear antibody, Anti-dsDNA, ssDNA

INTRODUCTION

The regimens required to treat TB are a challenge to administer. The most commonly used anti-TB antibiotics, all developed in the mid-20th century, remain the mainstay of therapy today. For the first time in over 40 years, 2 new anti-TB antibiotics have recently been approved for treatment.[2] Anti-TB regimens will vary depending on the stage and anatomic location of the infection, the immune status of the host, the age of the host, the presence of comorbidities, the development of toxicities, drug-drug interactions, and resistance patterns of the bacterium. Resistance is on the rise and often requires the administration of novel antibiotic combination strategies that have undergone limited testing in clinical

trials. The prolonged duration of therapy required to eradicate the organism represents an additional challenge. With respect to latent TB infection, shortened treatment duration strategies have recently been developed to minimize the adverse effects of antibiotics and maximize patient compliance.[3] The anti-TB drugs are associated with potential toxicities ranging from mild to life-threatening.[4] Patients must be educated about early signs and symptoms of drug toxicity, instructed about when to discontinue their use, and seek immediate evaluation should they occur. There are detailed guidelines for assessing treatment responses, patient monitoring, adverse drug reactions, and management.[5][6] There are several patients, on long term ATT regimens, with complaints of rash and/or arthralgia atfollow up visits. The etiology of these presentations present as a diagnostic dilemma to the physician i.e. whether the symptoms have been caused as a minor and single side effect of the drug or caused in thebackground of Drug Induced Lupus Erythematosus. Moreover, no existing literature could be found that studied the comparative antibody profiles of patientsdeveloping rash due to DILE and those developing rash due to other aetiologies Thus, an objective distinction based on the antibody profiles is essential to aid physicians.

MATERIAL AND METHODS

1 Study Area

This hospital based open, single-centered, open-label cross-sectional retrospective study was conducted in the Rheumatology OPD with the collaboration of Department of Biochemistry of Medical College, Kolkata, West Bengal, India.

2 Ethics Statement

The study was approved and permitted by the institutional ethics committee for patient care and use of laboratory and started after obtaining the written consent from the concerned ethics committee.

3 Calculation of sample size

The present study was conducted between March 2015 and November 2023. Sample size was calculated Using Cochrane Formula,

$n = (Z^2pq)/e^2$

n is the required sample size

Z = 1.96 for a confidence interval of 95%

p = proportion of the population with DLE = 0.02%

q = 100 - p = proportion of the population with negative Anti-Histone antibody in DLE=0.02%

e is the acceptable margin of error which is 5%

Hence, n = 10.92 = 11 [7]

By stratified random sampling the 11 study population consisted of patients who had been diagnosed with having DLE and rash di9sappear after discontinuation of ATT.

5 Diagnostic criteria of DLE [7]

Table 1: Criteria for the diagnoses of DLE

The patient has one or more clinical symptoms of SLE (eg, arthralgias, rash, fever, etc)
The patient had no history of SLE before using the culprit drug
The drug was taken anytime from 3 weeks to 2 years prior to the appearance of symptoms
Antinuclear antibodies are present
Clinical improvement is rapid when the drug is discontinued, whereas antinuclear antibodies and other serologic markers slowly decrease toward more normal levels

4 Collection of study population

After collection of Clearance from Institutional Ethics Committee, 10056 patients were taken from the Out Patient Departments in whom Isoniazid has been discontinued by the physician due to adverse effects such as arthralgias, rash, fever, etc but had no history of SLE before using the culprit drug. After48 hours of stoppage of drug, telephonic follow up of patients on status of rash and/or arthralgia and/or myalgia was taken with proper informed written consent for the study. Among these only 11 patients having disappearance of rash within 48 hours of discontinuation of the culprit drug were considered as study population of the present study. But those being known cases or having positive family history of autoimmune disease were not included in the study and who have known co-morbidities excluded from the present study.

5 Collection of samples from study population

Peripheral venous blood was drawn under aseptic precautions from all participants. All serum samples were stored at (-70^{0}C) and kept under these conditions until chemical analysis was performed. All parameter assays should be done as soon as possible.

6 Detection of ANA

ANA was detected by indirect immunofluorescence using Hep-2 as substrate, anti-dsDNA by antigen strips coated with dsDNA isolated from salmon testes, and anti-Smith (antiSm), and anti-U1-ribonucleoprotein (U1-RNP) by affinity chromatography using bovine and rabbit thymus. All assays were performed as stated in the manufacturer's product insert. To analyze the serum samples at the same dilutions, we used the starting dilution of 1:40 as stated in the Euroimmun package insert. All samples were read on a Nikon Eclipse 400 microscope at ×200, independently by 3 board-certified medical technologists with 2 to 8 years' experience reading IFA. The technologists were blinded to sample classification and each other's readings. Owing to the unique cell line used for the Immuno Concepts HEp-2000 assay, the 3 medical technologists were trained in performing and reading the slides by representatives of Immuno Concepts.

7 Analysis of Anti-dsDNA

The Anti-dsDNA-NcX ELISA microtiter plates (Nunc, Roskilde, Denmark) were coated at 4°C first with a 0.1 µg/ml concentration of an ultrapure nucleosome preparation from calf thymus (free of Scl-70, histone H1 and other nonhistone components) [8] in sodium carbonate buffer for 3 hours, followed by a 1.5 µg/ml concentration of highly purified, native dsDNA isolated from calf thymus in sodium carbonate buffer overnight. After being washed with 0.05% phosphate-buffered saline (PBS)-Tween 20 (vol/vol) and blocked for 2 hours with 0.1% PBS (wt/vol) casein, sera diluted 1:200 in 0.1% PBS (wt/vol) casein were added and allowed to react for 30 minutes. Bound antibodies were detected by use of antihuman immunoglobulin G peroxidase conjugate (EUROIMMUN Medizinische Labordiagnostika AG) and stained with tetramethylbenzidine (EUROIMMUN Medizinische Labordiagnostika AG) for 15 minutes. All steps were performed at room temperature. The optical density was read at 450 nm using an automated spectrophotometer (Spectra Mini; Tecan, Crailsheim, Germany). A highly positive index patient serum was used to generate a standard curve consisting of three calibrators (10, 100 and 800 international units (IU)/ml). IU were calculated for all samples using this three-point standard curve. The cutoff was optimized either by receiver operating characteristic (ROC) curve analysis (maximal sum of sensitivity plus specificity) or by predefined specificities of 98% and 99%. Commercially available anti-dsDNA ELISA, antinucleosome ELISA, CLIF and Farr assays (all from EUROIMMUN Medizinische Labordiagnostika AG) were used as reference assays and were performed according to the manufacturer's instructions.

8 Analysis of serum C3 and C4

C3 & C4 by immunonephelometry on the Atellica® NEPH630 System, BN II system and BN ProSpec® using antisera to human C3 or C4 with interassay CV 2.6% and intra-assay CV 1.9%.

9 Determination of ssDNA

Determination of Anti-ssDNA IgG by Anti-ssDNA IgG ELISA kit and was based on binding of ss-DNA antibody (IgG) from serum samples to ss-DNA immobilized on microtitre well having cutoff < 20U/ml with interassay CV 6.8-8.5% and intra-assay CV 5-6.5%.

10 Analysis of anti histone antibody

Semiquantitative analysis of anti histone antibody in serum was done by using ELISA considering 0.1 Unit is negative and >2.5 Unit is strongly positive.

11 Statistical analysis

Data were entered using Microsoft Excel 2007. Then the data for biochemical analysis was subjected to standard statistical analysis such as Student's t test using the Statistical Package for Social Science (SPSS) 27 software. For all tests 'p' value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

RESULT

1 Biochemical and anthropometric variables DLE population –

Baseline personal profile and clinical details of the study population are shown in Table 2.

Out of the 10056 patients whom Isoniazid has been discontinued by the physician due to adverse effects but had no history of SLE before using the culprit drug 11 (0.11%) had DLE. The study group of DLE comprised 6 females (54.6%) and 5 males (45.4%) with a female: male ratio almost 1:1. The patients' age range was 50-70 years with a mean age at presentation being 63.6 years. The most common age group was the fifth to sixth decade (58%).

Table-2: Biochemical and anthropometric variables DLE population

Study population (n = 11)	
Characteristics	**Number of participants**
Age in year	
<50	0 (0)
50-54	2 (18.2)
55-59	7 (63.6)
60-64	1 (9.1)
65-70	1 (9.1)
>70	0 (0)
Gender	
Male	5(45.4)
Female	6 (54.6)
Demographic data	
Urban background	6(54.6)
Rural background	5(45.4)
BMI (Kg/m^2)	23.8±2.8

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables

2 Serological profile of the study population-

In serological profile in selected study group, it was found that among 95% patients have positive anti-histone antibody and near about 90% have antinuclear antibodies. Almost all patients were positive for anti-ssDNA. But anti-dsDNA was rare and serum concentration of C3 and C4 were normal.

Table3: Serological profile of the study population (n =11)

Serological profile	**Observations**
Antihistone antibodies	96.7%
Anti-ssDNA	Present
Anti-dsDNA	4 (36.6%)
C3/C4 levels	Normal
Antinuclear antibodies	10(90%)
Antiphospholipid antibodies	3 (27.2%)
Antineutrophil cytoplasmic antibodies	8 (72.7%)

Data are expressed as numbers (group percentages in parentheses) for categorical variables

3 Generalized biochemical profiles of DLE patients–

Biochemical parameters of DLE patients such as ANA profile and anti-dsDNA are shown in the Table 3 and ANA Hep2 pattern in figure 1.

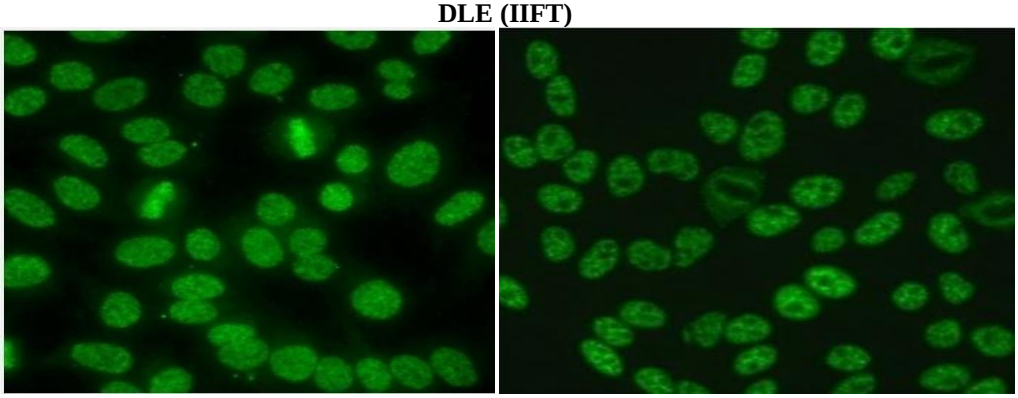
Table 4: Generalized biochemical profiles of DLE patients (n = 11)

ANA Hep2 (IIFT)	
4+ Homogenous	5 (45.4%)
3+ Coarse speckled	6 (54.6%)
ANA profile	
RNP/Sm +++	0(0%)
RO 52 +++	1(9.1%)
SSA +++	1(9.1%)

Data are expressed as numbers (group percentages in parentheses) for categorical variables; RNP - Ribonucleoprotein; ANA - Antinuclear antibody; Sm-Smith; Anti-CCP - Anti-Cyclic Citrullinated Peptide; RA Factor - Rheumatoid arthritis Factor Ro52/SSA - Sjogren Syndrome related antigen A

Figure 1: ANA Hep2 patterns of DLE performed by IIFT -

DLE (IIFT)



HOMOGENOUS PATTERN **COARSE SPECKLED PATTERN**

DISCUSSION

Drug-induced lupus may develop a few weeks to several months after starting the drug, which may make the diagnosis difficult. Further, it is not possible to differentiate DIL from SLE based on clinical features alone, although DIL tends to be milder and renal or CNS involvement, vasculitis, leukopenia, and pericarditis are rare.

Lupus-like symptoms with the exclusion of other autoimmune disorders and the resolution of symptoms with the withdrawal of medications suggest the diagnosis of DIL.[9,10] Hydralazine induced lupus is frequently manifested by arthralgia, myalgia, fever, rash (malar rash is common), hepatosplenomegaly, lymphadenopathy, and pleuritis. Rare cases of glomerulonephritis, neuropsychiatric manifestations and pericarditis have been reported. While arthralgia, myalgia, fever, and pleuritis are common in procainamide induced lupus, rash and lymphadenopathy are less common, and glomerulonephritis or CNS involvement is rare. Minocycline induced lupus is usually characterized by fever, arthralgia, arthritis, rash, and rarely pneumonitis and cutaneous vasculitis. Anti-TNF agents, which are the mainstay of treatment of rheumatoid arthritis now, have been frequently reported with positive autoantibodies as well as drug-induced lupus. While there is a high incidence of positive autoantibodies including ANA and Anti-dsDNA in patients treated with anti-TNF agents (up to 50%), only a few of those patients develop DIL (less than 1%).[11]

Skin rash is one of the most common clinical presentations of drug-induced lupus. The pathological examination of the biopsy from the skin rash in patients with DIL is similar to those with SLE.

So, laboratory evaluation is crucial but may not always be able to differentiate DIL from SLE. Cytopenias are less common, and if present, are mild. Methyldopa has been associated with hemolytic anemia while a positive coombs test has been reported with methyldopa, procainamide, and carbamazepine.

Autoantibody evaluation reveals a positive ANA, usually in a homogenous pattern, although the coarse speckled pattern has been reported. Anti-histone antibodies are present in 96.7% of cases of DIL, but in previous study, it was shown that their utility in differentiating DIL from SLE is limited given their positivity in up to 75% of cases of SLE.

Like other [7] Anti-dsDNA antibodies are seen in less than 5% cases of DIL. But in this previous study it was found especially secondary to anti-TNF agents and interferon-alpha. Rare cases of positive Anti-dsDNA have been reported in DIL secondary to minocycline and isoniazid.

Antiphospholipid antibodies including anticardiolipin antibodies and lupus anticoagulant have been reported in DIL secondary to chlorpromazine, procainamide, quinidine, and interferon-alpha. These are rarely associated with thrombotic events.

Antineutrophil cytoplasmic antibodies (ANCA) especially P-ANCA or atypical-ANCA have been reported in DIL secondary to minocycline, hydralazine, propylthiouracil, methimazole, and anti-TNF agents. Notably, up to 20% of patients with SLE can also have a positive ANCA.

Other autoantibodies against antigens including smith, ribonuclear protein (RNP), SCL70, centromere, Jo-1 are rare in DIL and may help differentiate DIL from other autoimmune disorders.

Laboratory evaluation shall also include evaluation of complements (C3 and C4), for INH induced DIL is indistinguishable from SLE.

Other autoantibodies against antigens including smith, ribonuclear protein (RNP), SSA, RO52 in our study are rare in DIL and that may help differentiate DIL from other autoimmune disorders.

Limitations of the study is that, it was conducted at a single center. It has a retrospective nature of the study design. The study has performed with low study population.

CONCLUSION

Serological profile of INH induced DIL in the study, it was found that among 95% patients have positive anti-histone antibody and 90% have antinuclear antibodies. Almost all patients were positive for anti-ssDNA. But anti-dsDNA was rare and serum concentration of C3 and C4 were normal.

Protecting patient privacy & autonomy-
The study was not utilized the full name or address of the patients nor the patient be denied of any service should they choose not to participate in the study. The test results were always shared with the patient or the caregiver as the case may be.As soon as the diagnosis of MM or relevant other disease was established the authors arranged for oncology referral so the treatment of the patient is not delayed.

Financial burden-
Cost for further investigations were borne by the authors on equal share basis and no additional expense were incurred by the participants for the tests. In case the patient needs to travel for such testing, the actual cost incurred was borne by the authors on a case – to case basis.

Conflicts of interest
There are no conflicts of interest.

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