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Role of anti-DFS70 antibodies in ruling out connective tissue disorders in Antinuclear Antibody (ANA) positive patients: A Cross-Sectional Study.

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Abstract

Background

Proteins are the target of Antinuclear Antibodies (ANAs), and the cell nucleus is where most nucleic acid antigens are found. The initial step in laboratory diagnosis for AARD is ANA detection. It is still difficult to comprehend the processes that underlie the production of ANAs in AARD and other chronic diseases of inflammatory.

Objectives

The purpose of conducting this study was to ascertain if isolated anti-DFS70 antibody positivity may be utilized to accurately rule out connective tissue disorders and to assess the value of diagnostics of anti-DFS70 antibodies in those individuals who are ANA-positive.

Materials and Methods

It was a cross-sectional, observational study. The study was carried out in the All-India Institute of Medical Sciences (AIIMS), Patna, Bihar, India. The study was conducted for 13 months. A total of 111 patients were enrolled.

Results

The average age of participants in the group of CTD was 40.1 ± 11.5 years, and CTD group age of participants 37.6 ± 12.6 years. Among all, 82 (73.9%) participants were female, and 29 (26.1%) participants were male. Isolated anti-DFS70 was seen in 16 patients, among them no participant had CTD, and all were non-CTD participants 16 (100%). Anti-DFS70 positive disease specific ENA was observed in 02 participants, and all the patients had CTD.

Conclusion

This study has revealed that isolated anti-DFS70 antibody positivity is quite specific for ruling out connective tissue disorders.

Recommendation

Routine testing for anti-DFS70 antibodies should be included in the diagnostic workup of ANA-positive patients to help effectively distinguish non-CTD cases and prevent unnecessary investigations.

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Introduction

Systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome (SjS), polymyositis/dermatomyositis, and systemic sclerosis are among the ANA-associated rheumatic diseases (AARD) that are characterized by antinuclear antibodies (ANAs) [1, 2]. Proteins are the target of ANAs, and the cell nucleus is where most nucleic acid antigens are found. The initial step

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in laboratory diagnosis for AARD is ANA detection. It is found to be very complex to know the processing of ANAs in AARD along with other chronic diseases of inflammatory. Numerous clinical and research labs have reported a somewhat common serum ANA pattern, which is depicted by the staining that is intense of mitotic chromosomes and dense, fine speckles in the nucleus. When human serum exhibits this pattern by indirect immunofluorescence (IIFA), a 70 kD band appears on immunoblotting. The dense fine speckled 70 antigen (DFS-70) was identified as the nuclear autoantigen. Chemiluminescence anti-DFS70 assay (CIA), along with the IIFA on Hep-2 cells were mainly observed for detecting these antigens. However, Bentow et al. [3] presented a unique immunoadsorption approach for anti-DFS70 detection.

It is mainly known that while measuring ANA, the positive output is known among the criteria for diagnosis for diseases of autoimmune, also these helps in activity of diseases, prognosis of disease, and sub-types of laboratory and clinical factors of the types; these all are considered as predictors for the development of pathology at the preclinical stage. It's also critical to note that autoantibody detection may occur before the disease's clinical symptoms appear. For instance, retrospective investigations have shown that high levels of ANA were found in the blood of 78% patients of SLE up to 10 years [4, 5].

A significant difference has been observed in the sensitivity and specificity among participants that has pattern of DFS in the test of ANA, was reported in the efficacy of diagnostics according a meta-analysis [6].

The purpose of conducting this study was to ascertain if isolated anti-DFS70 antibody positivity may be utilized to accurately rule out connective tissue disorders and to assess the value of diagnostics of anti-DFS70 antibodies in those individuals who are ANA-positive.

Methodology

Study Design

It was a cross-sectional, observational study. The study was carried out at the All-India Institute of Medical Sciences (AIIMS), Patna, Bihar, India, a tertiary care teaching and research hospital equipped with advanced diagnostic and immunology laboratories catering to patients from across eastern India. The study was conducted over 13 months, from July 2022 to July 2023.

Study Population

A total of 111 patients were enrolled in the study. Participants were selected from individuals who tested positive for ANA and attended the rheumatology and immunology outpatient departments during the study period. Consecutive sampling was used to include eligible patients until the desired sample size was reached. Inclusion criteria were: adults (≥18 years) who tested positive for ANA by HEp-2 indirect immunofluorescence with intensity ≥2+, and who received a thorough clinical evaluation by a rheumatologist, enabling accurate classification into CTD, also known as SARD/AARD, or non-CTD groups. Exclusion criteria were: individuals with a pre-existing CTD diagnosis who were not re-evaluated, and those without standardized classification or rheumatologist-confirmed diagnosis.

Data Collection

The study contained information on age, gender, and classification based on laboratory parameter results. During the data gathering procedure, structured forms and clinical records were employed to ensure accuracy and consistency. Each patient provided their informed consent before any data collection.

Study Procedure

The EUROIMMUN Germany kit was used for ANA IIF, and the test was performed at a 1:100 dilution in PBS-Tween, as per the kit guidelines. Stained BIOCHIP slides were graded from + to ++++ based on fluorescence intensity under a fluorescent microscope, with no fluorescence reported as negative. All those ANA positive samples which were included in study underwent full serological testing, including anti DFS70 and a full panel of disease-specific ENA autoantibodies The anti-ENAs test was performed using a profile of ANA along with DFS70-IgG kit (EUROIMMUN, Germany). The electronic file had information on the patients' demographics, ANA pattern, anti-ENAs test findings, and AARD diagnosis. SS, SLE, myositis, MCTD, SSc, drug-induced SLE, and SLE/SSc overlap were the types of AARDs.

Statistical Analysis

Microsoft Excel and SPSS version 24.0 were used to assemble and analyze the study's data. Continuous variables were shown as mean±standard deviation (SD), whereas

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variables that was categorical were shown as percentages or the number of participants (n).

Ethical Clearance

Informed consent was taken from all participants after getting ethical clearance from the Institutional Ethics Committee of the All-India Institute of Medical Sciences (AIIMS), Patna, Bihar, India.

Results

Table 1 shows study participants demographics at baseline. The average age of participants in the CTD group was 40.1 ± 11.5 years, and CTD group age of participants 37.6 ± 12.6 years. Among all, 82 (73.9%) participants were female, and 29 (26.1%) participants were male.

Table 1. Demographics of Study Participants

Parameter	Total (n = 111)	CTD Group (n = 38)	Non-CTD Group (n = 73)
Age (in years)	38.5 ± 12.2	40.1 ± 11.5	37.6 ± 12.6
Gender			
Female	82 (73.9%)	30 (78.9%)	52 (71.2%)
Male	29 (26.1%)	8 (21.1%)	21 (28.8%)

Isolated anti-DFS70 was seen in 16 patients, among them no participant had CTD, and all were non-CTD participants 16 (100%). Anti-DFS70 positive disease specific ENA was observed in 02 participants, and all the patients had CTD. And, anti-DFS70 negative patients had 93 participants in total, 36 (38.7%) of patients had CTD, and 57 (61.3%) patients had non-CTD.

Table 2. Status of Anti-DFS70 of Study Participants

Status of Anti-DFS70	CTD (n, %)	Non-CTD (n, %)
Isolated anti-DFS70 (n=16)	0 (0%)	16 (100%)
Anti-DFS70 + disease-specific ENA (n=02)	2 (100%)	0 (0%)
Anti-DFS70 negative (n=93)	36 (38.7%)	57 (61.3%)

The specificity of the test was found to be 97.6%, and sensitivity was 5.3%. Table 3 depicts the performance of the diagnosis.

Table 3. Performance of the Diagnosis

Parameter	Value (%)
Sensitivity	5.3
Specificity	97.6
Positive Predictive Value	11.1
Negative Predictive Value	98.6

Discussion

The current investigation assessed antibodies of anti-DFS70 diagnostic utility in patients with ANA-positive as well as their function in ruling out CTDs. 16 (14.4%) of the 111 ANA-positive patients exhibited isolated anti-DFS70 positivity; interestingly, none of these patients had a CTD

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diagnosis. The idea that isolated anti-DFS70 antibodies were predicted to be an AARD marker that is excluded is supported by this finding.

In an ANA test, the antibody that is anti-DFS70 manifests as a pattern of DFS and targets the DFS70 protein [7]. The DFS70 protein is thought to be a transcription of stress-activated co-activator since it increases the expression of genes linked to cancer, the stress response, and antioxidants in a variety of cell types. Nevertheless, little is known about the role physiologically along with DFS70 importance in conditions which are of non-diseased [8].

Even while the majority of specialists advise utilizing antibodies of anti-DFS70 which are monospecific to rule out SARD, we discovered that anti-DFS70 antibodies by themselves, without concurrent autoantibody testing, might still achieve high specificity in patients who present with a test of ANA which is positive [9]. Levels of antibody of Anti-DFS70 in ostensibly healthy people have been observed to be as low as 30%. Antibodies of anti-DFS70 were seemingly observed in 3.8–37.3% of people with a positive ANA test and in 6.4–42.7% of people with non-SARD and a positive ANA test. While individuals without non-SARD who test positive for ANA may possess anti-DFS70 antibodies, the prevalence of these antibodies is likely insufficient to provide high sensitivity for reliably identifying non-SARD cases.

With a negative predictive value (NPV) of 98.6% and a high specificity of 97.6% for ruling out CTD, isolated anti-DFS70 was in line with earlier research findings published in the literature. A study by Leefang et al. reported that disease and its prevalence were associated with specificity or sensitivity of accuracy of diagnostic tests [10]. Thus, specificity and sensitivity both are considered as factors that can affect prevalence of the disease and the accuracy of diagnosis which can further lead to change in prevalence of the disease [11].

Anti-DFS70 antibodies should not be utilized as the only diagnostic method for confirming or ruling out CTDs, as the test's low sensitivity (5.3%) and positive predictive value (11.1%) highlight. Rather, it ought to be evaluated in light of further serological data and clinical observations.

Generalizability

The findings of this study may apply to similar tertiary care settings and populations with comparable demographic and clinical profiles but should be validated in larger, multi-center cohorts.

Conclusion

According to the study's findings, in patients with ANA, isolated anti-DFS70 antibody positivity is quite specific for ruling out connective tissue disorders. Its existence, particularly in the absence of other illness-specific autoantibodies, can aid in ruling out autoimmune rheumatic disorders and preventing needless tests, even though its low sensitivity prevents it from independently confirming the absence of disease.

Limitations

The main limitation of this study was the small number of patients, which might affect the efficiency and the duration of time.

Recommendations

Large-scale studies are necessary to validate these observations and enhance our understanding of role of anti-DFS antibodies.

Source of Funding

This study was conducted without any external funding or financial support.

Conflict of Interest

The authors declare that there is no conflict of interest related to this study.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

List of Abbreviations

AARDs- ANA-associated rheumatic diseases
CTD- Connective tissue disease
AIIMS- All India Institute of Medical Sciences
ANA- Antinuclear antibodies
SLE- Systemic lupus erythematosus
MCTD- Mixed connective tissue disease
IIF- Indirect immunofluorescence
DFS70- Dense fine speckled 70 antigens
CIA- Chemiluminescence anti-DFS70 assay

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