ORIGINAL RESEARCH ARTICLE

Retrospective Assessment of Antinuclear Antibody by Indirect Immunofluorescence Microscopy and Immunoblot in Patients with Polyarthritis

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Abstract

Introduction: Detection of antinuclear antibody (ANA) by immunofluorescence assay (IFA) is the method of choice for screening autoimmune polyarthritis, where the different patterns are etched in the cellular components as per the group of antibodies present in the patient. Like all other screening tests, it has its sensitivity and specificity, and the final confirmation is done by doing a multispot immunoblot testing, where the specific antibodies against a specific antigen is detected.

Aims and objectives: The study aimed at finding out the sensitivity and specificity of IFA with respect to immunoblot assay and to detect whether any pattern can be detected to attribute to false-positive and false-negative cases.

Materials and methods: The conducted study is a retrospective analysis of 100 reports available from the Laboratory Information System. Patients referred by rheumatologists with clinical polyarthritis prescribed for both ANA by IFA and immunoblot are taken for the study. Sensitivity and specificity of ANA by IFA is calculated taking immunoblot as confirmatory gold standard.

Results: The sensitivity and specificity were calculated as 80 and 84.3%, respectively, in polyarthritis cases and anticell (AC)-1 and AC-4 were implicated in most of the false-positive and false-negative cases.

Conclusion: Antinuclear antibody by IFA needs confirmation by immunoblot for antibody profile characterization. AC-1 and AC-4 if detected on IFA mandates for immunoblot as most of the false positives and negatives are implicated with these patterns.

Keywords: Antinuclear antibody, Immunoblot, Immunofluorescence microscopy, Polyarthritis.

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Introduction

Antinuclear antibody (ANA) testing by immunofluorescence microscopy is a screening test for systemic autoimmune rheumatic diseases (SARDs) involving a spectrum of diseases like systemic lupus erythematosus (SLE), drug-induced lupus erythematosus (LE), polymyositis, dermatomyositis, rheumatoid arthritis, and Sjogren's syndrome. Patients suffering from polyarthritis present with painful sensation in bones, fibrous tissues, and muscles around the joints. Though guidelines for treatment is available, most of the patients with SARD do not achieve clinical remission. Rather, the disease results into significant disability of the patient and reduced quality of life.[1]

So far prevalence of SARD is concerned, the occurrence of the diseases is relatively less common but, combinedly, they affect up to 5% of the global population[2] and in a populous country like India the numbers are significant to address it as one of the major health problems gaining importance with time. In recent years, the prevalence is reported to increase due to many reasons. Increasing population of aged persons, use of certain drugs, and exposure to different chemicals and toxins in environment are some of the contributing factors for such diseases.[3,4]

Naturally, early diagnosis of the disease is very important to start the treatment so that life-threatening consequences can be minimized. For this, a biomarker is extremely useful SARDs are considered as autoimmune disease, where ANAs are produced against antigens in the cell nucleus.[5] Antinuclear antibody is present in serum and was reported to be a biomarker in the entire spectrum of SARDs.

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There are different methods of ANA assay. Among these methods, indirect immunofluorescence assay (IFA) using human epithelial cell tumor (HEp-2) cell line is the most effective method. It etches different patterns in the nucleus and cytoplasm of

Hep 2 cells which are correlating to a group of autoantibodies present in the patient serum. As an example, for an ANA pattern of nuclear homogeneous, the ICAP code designated is anticell (AC)-1 and antibodies are against ds-DNA, ss-DNA, nucleosomes, histones, and disease correlating are SLE, drug-induced LE, juvenile idiopathic arthritis.[6]

Antinuclear antibody IFA can detect wide-ranging autoantibodies. The method is highly sensitive and there is concurrently staining patterns and titers can be detected.[7] Nevertheless, the ANA IFA has some drawbacks also. Adequate standardization is not possible for recognition of the pattern and it is largely dependent on the abilities of individual investigators. Thus significant variations within a laboratory and among several laboratories have been observed in interpreting results. To overcome this problem, there exist other alternative methods, which are enzyme-linked immunosorbent assays, line immunoassays, and multiplex bead assays.[8,9]

Enzyme immunoassay (EIA) commercial kits were manufactured to detect ANA, using HEp-2 nuclear extracts and purified or recombinant antigens. The studies comparing between ANA IFA and ANA EIA have yielded with discordant results.[10,11] Antinuclear antibody testing by immunoblot is reported as confirmatory test. Here purified antigens are impregnated on a nitrocellulose paper, which on incubation with diluted patient sample detects autoantibodies present in patient serum against specific antigens.

Aims and Objectives

In this backdrop, this study aimed at finding out the diagnostic accuracy of pattern of polyarthritis by ANA IFA with respect to immunoblot assay. The objectives of the study can be considered as the following:

- To visualize and find the patterns of ANA which are mainly etched in polyarthritis.
- To find the sensitivity and specificity of IFA with respect to immunoblot assay.
- To detect whether any pattern attributes more to false-negative and false-positive cases.

Materials and Methods

This study is a retrospective, cross-sectional in nature using secondary data from the Laboratory Information System (LIS) Records of Unipath Specialty Laboratory, which is a large NABL accredited laboratory. No ethical permission was required as no human subjects were handled directly. Only secondary nonidentifiable data were used. The subjects were not identified by name but by the lab reference number. Thus, anonymity and confidentiality of the subjects were preserved. This is in accordance to the Declaration of Helsinki guidelines. However, a written permission was obtained from the Director of the Laboratory. The study involved a retrospective analysis of 100 reports available from the LIS. Reports of patients suffering from clinical polyarthritis and were referred by rheumatologists for detection of ANA by both IFA and immunoblot method were included in the study. Data were generated by comparing the ANA IFA reports along with their corresponding immunoblot reports of the individual patients. Data were tabulated and analyzed by applying appropriate formula. Sensitivity and specificity of ANA by IFA is calculated taking immunoblot as confirmatory gold standard.

Table 1: Distribution of study population according to gender

Sl. no.	*Gender*	*Number (%)*
1	Male	12 (13.18%)
2	Female	79 (86.81%)

Results and Discussions

Reports of a total of 100 patients were collected from the laboratory database during a period of 12 months. Among these patients, three were untraceable due to some payment issue and six reports were not comparable due to some rare patterns. Thus, reports of 91 samples were ultimately taken into account. Distribution of study population according to gender and age is shown in Table 1 and Figures 1 to 3, respectively. The data of Table 1 clearly show a female preponderance.

Affected age-group was 31–40 years for female patients and 41–50 years for male patients. Minimum age found for male and female patients is 18 and 11 years, respectively, whereas maximum age was 80 and 79 years, respectively. So, there is a clear indication of affection of females at a younger age.

Women have a set of XX sex chromosomes, while in men, it is XY sex chromosomes. The X chromosome is larger than the Y chromosome in size, containing a greater number of genes than the Y chromosome.[12] The X chromosomes contain approximately 800–900 genes making up for 5% of total DNA in human cells, whereas the Y chromosomes contain approximately 50–60 genes coded to provide instructions for proteins and this comprises 2% of DNA in human cells.[13,14] The X chromosome also has for a greater amount of immune-related genes and immune regulatory genes, which participates in and induces immunological responses in the body.[15] The larger number of genes from the X chromosome creates a far great possibility of a greater number of mutations happening. This puts women at a greater risk for the development of autoimmune diseases solely due to women having two X chromosomes. The presence of two X chromosomes creates a more double predilection of genes present on the X chromosome and thus, predisposes the female to autoimmune diseases.[16] The fluctuations in hormone levels in female individuals during puberty enhance the risk of development of autoimmune diseases,[16] which explains the affection of females at a younger age. Hormonal changes occurring during the postpartum period lead to an enhanced incidence of autoimmune diseases, such as rheumatoid arthritis[17] adding to female predilection of autoimmune diseases. In a study by Agmon-Levin et al., it was found that in the postpartum period, there is a significant increase in the incidence of rheumatoid arthritis cases with an incidence rate ratio of 1:7, in 2 years after delivery.[7] These studies are in accordance with the findings of this study concerned.

Of 91 chosen study subjects, ANA was positive in 40 cases by ANA IFA and a maximum of 16 were noted in AC-1 (nuclear homogeneous) pattern and a single case of AC-6 (multiple nuclear dots) constituted the least as shown in Table 2. Distribution of study population according to positivity of ANA by two methods is presented in a 2 x 2 contingency (Table 3). Immunofluorescence assay detected 40 cases as positive, whereas true positivity was found in 32 cases by immunoblot. Out of 51 negative cases detected by IFA, 43 were reported as true negative by immunoblot and thus the sensitivity of ANA IFA in cases of polyarthritis was calculated as 80% and specificity as 84.3% as shown in Table 4. Data analysis also revealed that false-positive ANA IFA was found in AC-1 (four cases),

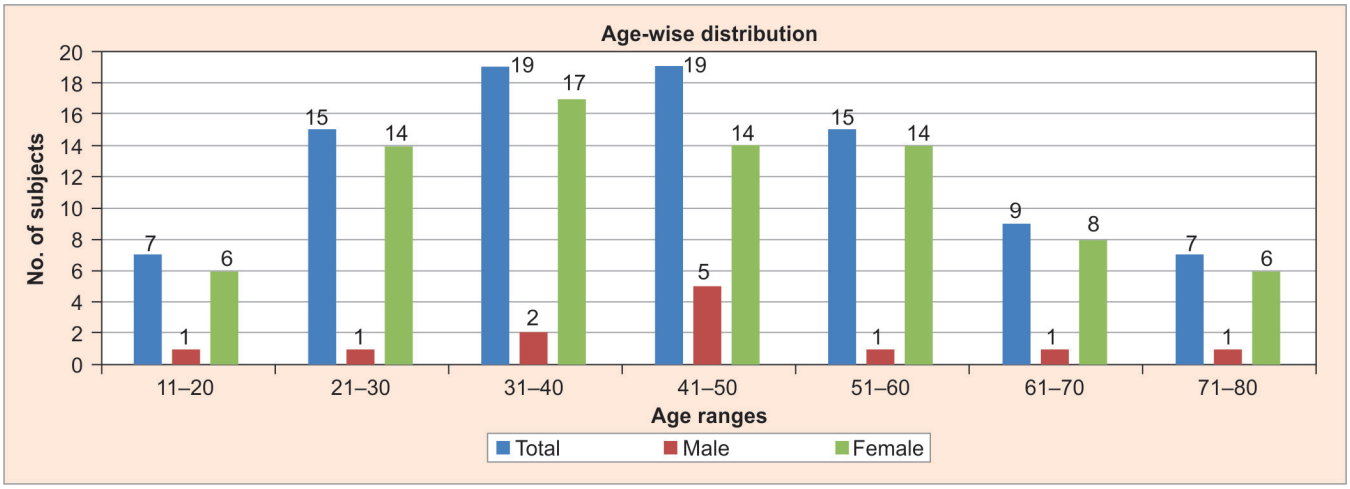

Fig. 1: Distribution of study population according to the age-group

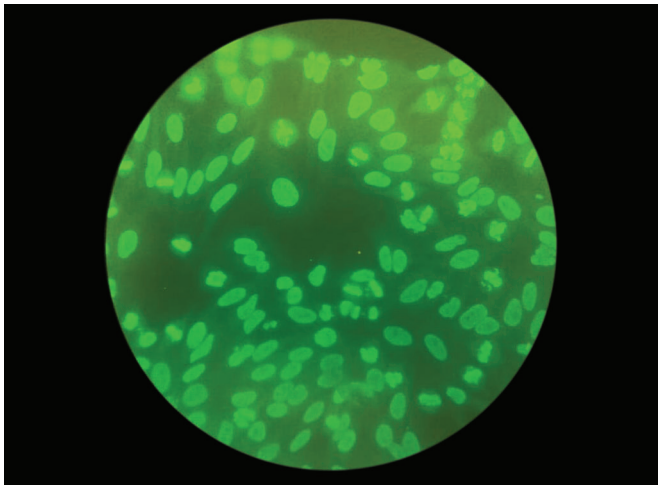

Fig. 2: Nuclear homogeneous (AC 1 pattern) under immunofluorescence microscopy

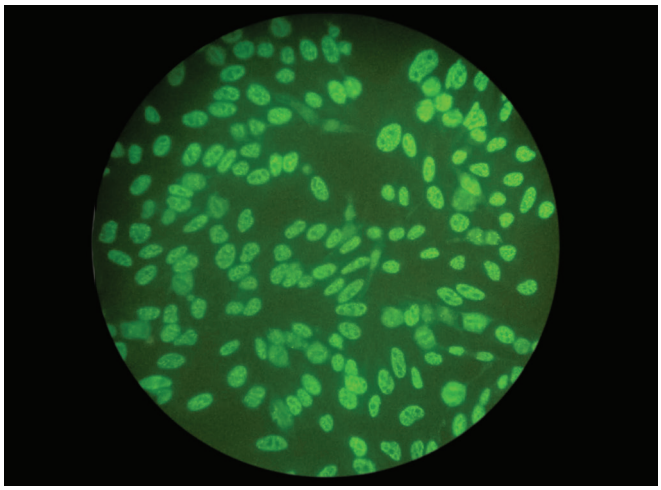

Fig. 3: Nuclear fine speckled (AC 4 pattern) under immunofluorescence microscopy

AC-4 (one case), AC-9/10 (two cases), and AC-8 (one case). False-negative ANA IFA was found in AC-1 (one case) AC-4 (three cases), AC-5 (two cases), AC-15 (one case), and AC-19 (one case). Thus, AC-1 pattern is mostly found in false-positive cases, whereas AC-4 pattern is most common in false-negative cases. By extensive literature search, no such similar study was found.

Table 2: Distribution of ANA patterns in polyarthritis patients

Sl. no.	*ICAP code (patterns)*	*No. of cases*
1	AC 1 (Nuclear Homogeneous)	16
2	AC 3 (Centromere)	2
3	AC 4 (Nuclear Fine Speckled)	4
4	AC 5 (Nuclear Coarse Speckled)	7
5	AC 6 (Multinuclear dots)	1
6	AC 21 (Mitochondrial)	2
7	Mixed pattern	8
8	Negative	51

Table 3: Comparison of ANA IFA taking ANA immunoblot assay as gold standard

Sl. no.	*Parameters*	*ANA IFA positive*	*ANA IFA negative*
1	ANA immunoblot positive	32 (true positive)	8 (false negative)
2	ANA immunoblot negative	08 (false positive)	43 (true negative)
	Total	40 (43.95%)	51 (56.04%)

Table 4: Sensitivity and specificity of ANA IFA taking ANA immunoblot assay as gold standard in polyarthritis cases

Sl. no.	*Parameters*	*Results*
1	Sensitivity of ANA IFA	80%
2	Specificity of ANA IFA	84.3%
3	Positive predictive value of ANA IFA	80%
4	Negative predictive value of ANA IFA	84.3%

Conclusion

Antinuclear antibody by IFA needs confirmation by immunoblot for antibody profile characterization. AC-1 and AC-4 if detected on IFA mandates for immunoblot as most of the false positives and

negatives are implicated with these patterns. Similar studies in relation to different autoimmune diseases can evaluate the ANA IFA and its effectivity in the plethora of autoimmune diseases.

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