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Antinuclear Antibody (ANA) Pattern Distribution & Clinical Relationship in a Tertiary Care Centre

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Abstract

Introduction: Antinuclear antibodies (ANA's), are one of the most important tests in immunology, although very informative it faces major challenges. Confronting a positive ANA in a patient without clinical disease but consistent symptoms, is a game changer for the treating physician. This study aims to determine the rate & pattern of ANA positivity and understand its distribution pattern and clinical diagnosis in cases evaluated at a tertiary care centre.

Materials and Methods: This observational analytical study was carried out for a period of one year. A single serum sample was collected from patients ranging from age groups 10 to 65 yrs suspected to have an autoimmune disease. Samples were tested by indirect immunofluoresence (IIF) and patterns were recorded and analysed.

Results: Among the 150 tests done, 15 (10%) cases were ANA global test positive, with a mean age was 40 and greater positivity among females patients 16 (17.3%) (p-value <0.001). The most common pattern encountered was the nucleoplasm fine granular variety 10 (40%) followed by nucleoplasm coarse granular 5 (20%).Various other patterns were also observed with a predominance of cell nuclei homogenous pattern 4 (16%). ANA positivity was observed in 95% of Systemic lupus erythematosus (SLE) and mixed connective tissue disorders (MCTD) and in 25-70% of the cases with Sjögren syndrome, Systemic sclerosis (SS) and Rheumatoid arthritis (RA). Some ANA's showed weak fluorescence detectable before the actual onset of clinical symptoms being apparent and was particularly seen in suspected cases of SLE.

Conclusion: The rate and distribution of pattern types correlated well with the autoimmune condition and even borderline or weak intensity fluorescent patterns should be reported and the patients having them should be followed-up regularly.

Keywords: Antinuclear antibodies; Indirect immunofluoresence; Systemic lupus erythematosis.

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Introduction

Autoantibodies are immunglobulins formed directly against autoantigens that are known as endogen antigens. Autoimmune disease is an occasion that the body begins a fight against it's own cells and tissues. The unusal antibodies that are directed against structures within the cell nuclei are called as anti-nuclear antibodies (ANA). Some of the autoimmune diseases are Raynaud's

phenomenon, SLE, RA, scleroderma and multiple sclerosis.[1-3]

Autoantibody detection in serum samples plays important role in diagnosis and in follow-up of autoimmune diseases for the last 50 years. An indirect IIF kit is available as a screening test.[3,4] The American College of Rheumatology (ACR) supports ANA detection by IIF as gold standard for ANA testing.[5] Thus ANA tests have remained important because some of the immunofluorescence patterns are extremely helpful in diagnosis and in some cases the clinician needs the support of IIF results.[6-9] Besides this, detection of ANA positivity in healthy individuals in the community shows the need for studies to be conducted on prevalence.[3,4] This study helps us determine the rate of ANA positivity and patterns of positive specimens and understand the ANA distribution pattern and clinical diagnosis in cases evaluated at a tertiary care centre.

Materials & Methods

This observational analytical study was carried out in the Department of Microbiology of a tertiary care centre for a period of one year from Feb 2017 to Jan 2018 after ethical clearance. A single serum sample was collected from patients (including inpatients and outpatients) ranging from age groups 10 to 65 yrs suspected to have an autoimmune disease. All patients suspected to have an autoimmune disease were included and those with diseases such as diabetes mellitus, hypertension, heart failure were excluded from the study.

An immunofluorescence kit used was from EUROIMMUN, Germany was used. The dilution protocol followed was 1:100. After collection of demographic and clinical details of the patients under study, their serum samples were subjected to the protocol for IIF testing. The slides were prepared following the recommendations of the manufacturer. The evaluations were done under a fluorescence microscope using 40X objective (Fig. 1 & Fig. 2). Positive and negative controls were incorporated in all evaluations. Fluorescence intensity was interpreted semi quantitatively based on negative control (0) and positive control (+3). Differences and associations between the variables were analysed by using Chi-square test. The results were evaluated within a confidence interval of 95%, and a *p*-value of less than 0.05 was considered statistically significant.

Results

Among the 150 tests done, 25 (16.6 %) cases were ANA global test positive. The mean age was 40 among the ANA-positive patients and was 46 in ANA-negative individuals. Amongst the patients, 58 (38.6%) were males and 92 (61.3%) were females. ANA was positive in 9 (36%) of the males and 16 (64%) of the females. Thus ANA positivity rate was significantly higher among females than males with ratio of 1:1.8 (*p*-value <0.001). The most common pattern encountered was the fine nucleoplasm granular variety 10 (40%) of SS-A/SS-B, nucleoplasm coarse granular 5 (20%) of nRNP/Sm. The other patterns observed were cell nuclei homogenous pattern 4 (16%) and nucleoplasm dotted pattern i.e., antibody against centromere 3 (12%) and antibody against nuclear dots i.e., 2–6 dots 2 (8%) cases and nucleoli positive Scl 70 pattern 1 (4%) (Table 1).

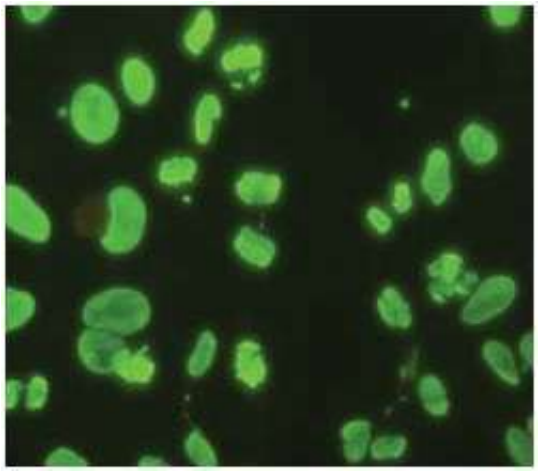


Fig. 1: Nuclei homogenous pattern under 40X

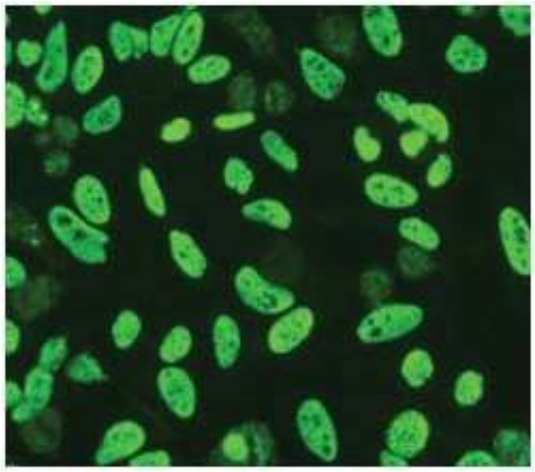
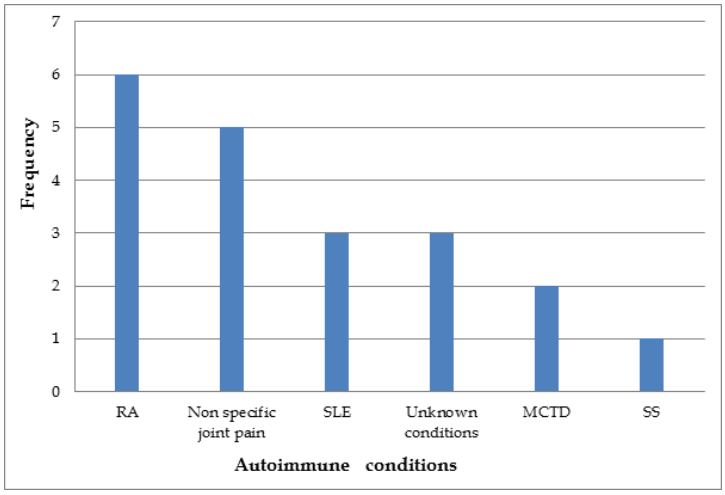


Fig. 2: Coarse granular nucleoplasm pattern under 40X

The most common autoimmune condition was rheumatoid arthritis (RA) 6 (24%), non specific joint pain 5 (20%), systemic lupus erythematosus (SLE) 3 (12%), unknown conditions 3 (12%), mixed connective tissue disorders (MCTD) 2 (8%) and systemic sclerosis (SS) 1 (4%) (Graph 1). ANA positivity correlated well clinically and was significantly higher in cases with rheumatoid arthritis with a *p*-value of <0.001 when compared to those with non specific joint pain and anemia than with SLE, MCTD or SS. ANA global test (IIF) results correlated well ANA profile test.

Table 1: Distribution of anti-nuclear antibody patterns in the study group

Anti-nuclear antibody patterns	Number of cases
Fine nucleoplasm granular variety of SS-A/SS-B	10
Nucleoplasm coarse granular variety of nRNP/Sm	5
Nuclei homogenous pattern	4
Nucleoplasm dotted pattern	3
Antibody against nuclear dots	2
Nucleoli positive Scl 70	1
Total	**25**



Graph 1: Distribution of various autoimmune conditions.

Discussion

The antibodies that target normal proteins within its own cell nuclei are called as anti-nuclear antibodies (ANA). Autoimmune diseases have various symptoms and findings. These may be classified as organ-specific and systemic autoimmune disorders. Some of these are insulin-dependent diabetes mellitus, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma and multiple sclerosis.[10]

At times patients are eager to know if he/she will develop that particular autoimmune condition based on the findings of a routine fluorescent testing. Thus an explanation for the frequency of ANA expression in the general population relates to intrinsic immunological disturbances among humans.[11] Most individuals with a positive ANA did not have an autoimmune disease per se and most also are unlikely to develop one.[12] But a follow up of such cases can help identify autoimmunity in the long run.

The SS-A/SS-B pattern turned out to be the most common pattern in our study showing a grade of 1+ or 2+ but at the same time 3 (30%) of the cases didn't present with any typical symptoms of autoimmunity. Though traditionally, ANA are detected by indirect immunofluorescence (IIF) performed on human epithelial cells (HEp-2), but this technique involves several stages or processes of visual identification of the staining pattern and serial titrations of positive sera followed by a second test in which autoantigen specificity is confirmed like the ANA profile test.[13,14] Recently, the American College of Rheumatology (ACR) proclaimed that ANA detection by IIF is still considered the gold standard.[15]

Our ANA prevalence was comparable to a study by Thomas *et al.* where the higher relative ANA prevalence in tertiary care was (16.0%) versus secondary care being (10.8%).[15] Due care must be exercised while interpreting these test by giving importance to higher titres and keeping in mind that cross reacting antibodies may reveal a positive test in low titres. This aspect is also valid when ANA levels tend to rise during a flare.[16] Low titers of autoantibodies are seen in healthy people, relatives of the autoimmune patients and patients having chronic inflammatory disease or cancer without any autoimmune base. These kinds of antibodies are usually in IgM type of low affinity and polyreactive. Li *et al*. suggests that the persistence of the positive ANA may be a part of the component of the normal immune response.[9]

Our study attributed a greater percentage of these antibodies among females (61.3%) similar to a study by Nevreste *et al*'s study where ANA positivity rate among the female study group was (77%).[16,17] The study concluded that it is probably the hormone profile, fetal microchimerism and certain strategic genes which are on the sex chromosomes that might play a role on this relationship.[18]

The most common patterns seen in our study were different from similar studies in Turkey were their predominant pattern was cell nuclei homogeneous (23%), nucleoplasm granular (22%), homogeneous-granular (15.5%) and nucleolar (13.5%). Guducuoglu *et al*. also reported 152 homogeneous, 96 nucleolar, 82 granular pattern out of 367 ANA positive patients.[16]The dominantly seen pattern was the homogeneous type (51.2%) and was followed by fine granular (6%), homogeneous/fine granular (6%) and homogeneous/nucleolar (6%) by Yılmaz *et al*.[7] Karakece *et al*. found that most frequent patterns in his study was the nuclear type (56.2%); fine and coarse granular, homogeneous and nuclear membrane, nucleolar (16.2%), mitotic (14%) and cytoplasmic (13.6%).[19]

According to our study, the most frequent four antigens were SSA (34.4%), SSA-SSB (16.8%), Scl70 (16%) and Sm/RNP (9.2%) respectively. Anti-Sm antibodies were mostly found in SLE patients. Similarly, Scl70 was found to be 100% specific for the diagnosis of systemic sclerosis. If SSA or/and SSB are detected, the result will direct us not only to diagnose Sjögren's syndrome but also identify subacute cutaneous SLE and neonatal lupus syndrome.[20] Some studies reported that some extractable nuclear Antigens (ENA's) especially anti-SSA/Ro and anti-SSB/ La antibodies can be missed on IIFA, although others demonstrated borderline fluorescence might contain these antibodies.[21] It should remembered that depending on the positive ANA pattern anti-ENA profile may be negative.

At times it was noted that ANA test was positive in connective tissue disorders, however, it can be added that ANA positivity is not absolutely diagnostic. Among the ANA positives that we had in our study, 5% of them were healthy individuals for some unknown reasons. These positives may be seen when sera is diluted 1:40 according to Tan *et al*.[22]

But however this phenomenon was evident even in dilutions of 1:100. It is wise to repeat the test after a 3–6 months period when the suspected symptoms of the disease disappear. A positive result in a repeat test would clinch the diagnosis, however a negative result could mean that the first positivity might have been due to a probable polyclonal B activation according to a report by Afsar *et al*.[6] As the intensity of fluorescence does not reflect the concentration of antibody, further studies are needed to elucidate whether grading of intensity is mandatory in interpretation.[23] To find out if any relationship exists between the intensity of fluorescence and the concentration of anti-nuclear antibodies a further testing by a quantitative enzyme linked immunosorbent assay to estimate the concentration of anti-ds DNA antibody can be done.

All these interpretations along with a good interaction with the clinicians to rule out drug induced ANA, autoimmune liver disease or thyroid disease, chronic hepatitis C infection or other recent viral infections is an indispensable component of confidential analysis and reporting.[15] Newer technologies like multiplex immunoassays, antigen microarrays, quantum dots and other fluorescent nanoparticles in the long run will enhance the clinical perspective of this condition.[15]

Conclusion

The rate and distribution of pattern types correlated well with most of the autoimmune conditions however borderline or weak intensity fluorescent patterns should not be ignored but reported and the patients having them should be followed-up regularly.

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