

Antinuclear Antibody Detection by Immunofluorescence Test: Insight from a Tertiary Care Hospital, North Western India**Jain Ruchi¹, Bithu Rameshwari², Yadav Manju³, Aggarwal Akriti*⁴, Lamba Anita⁵, Khandelwal Sonam⁶**¹Senior Demonstrator, Department of Microbiology, SMS Medical College, Jaipur²Senior Professor, Department of Microbiology, SMS Medical College, Jaipur³Associate Professor, Department of Microbiology, SMS Medical College, Jaipur^{4,5,6}Senior Resident, Department of Microbiology, SMS Medical College, Jaipur

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Corresponding author: Dr. Akriti Aggarwal

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Abstract:

Introduction & Objectives: Anti-nuclear Antibodies (ANA) are said to be linked with various autoimmune diseases. ANA detection by Indirect Immunofluorescence Assay (IIFA) using Hep 2000 cells is considered as gold standard method for diagnosis. ANA screening is a reliable and a quick way to detect immunofluorescent patterns linked to autoimmune disorders. Thus that being the aim of our study to identify the prevalence of ANA patterns amongst the various patients presenting in a tertiary care hospital in Jaipur, Rajasthan.

Material and Method: This is a retrospective study which included 3016 patients with either clinical suspicion of or suffering from autoimmune diseases attending a tertiary health care hospital in Jaipur, Rajasthan. ANA IFA test was performed with 1:80 dilutions of patient serum.

Results: Out of 3016 samples processed 596 samples (20%) were positive. Out of various ANA patterns observed, most common pattern seen was nuclear speckled (81.54%) pattern.

Conclusion: Autoimmune disorders are showing an increasing trend and ANA IFA is a promising tool for detection of antinuclear antibodies giving a clue for diagnosis of autoimmune diseases based on different ANA patterns.

Keywords: ANA, IFA, Fluorescent patterns.

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Introduction

Autoimmune diseases (AD) are an immunological response of the body to antigens of the self-leading to damage of tissues in the body. [1] Systemic Autoimmune Rheumatic Diseases (SARDs) constitute a major part of these AD having a complex pathology & patients presenting with very varied symptoms. [2] It mostly affects the middle aged to older population and mostly females [3], though some authors have reported a large no. of cases of connective tissue diseases in children too. Therefore, the etiology of ANA till date hasn't been fully understood.

A lot of risk factors are said to increase the predisposition to AD like genetics, exposure to physical & chemical agents though a very comprehensive review of the same isn't available. [4,5] These diseases are very disabling, crippling the life of the patient but the major problem being the lack of a timely diagnosis & one of the major reasons for that is the lack of definitive symptoms in early stages & second the laboratory diagnosis being complicated due to the need of specialized

tests to detect antibodies against self. [6] A large no. of tests is available but the IIFA being the gold standard for detection of ANA. The recent guidelines suggest the usage of the human laryngeal carcinoma cell line (HEp-2000) for detection of ANA. The reasons these cell lines are chosen are because they comparatively have a big nucleus and a smaller amount of cytoplasm thus helping in optimal patterns detection. [7]

Whenever a patient is suspected of having an AD, ANA is done as an initial diagnostic test. These are the antibodies which are detected in the blood having the ability to bind to certain structures in the cells nucleus. [8] Around 29 different types of HEp-2 cell IIFA patterns are recognized and are further classified into nuclear, cytoplasmic and mitotic subtype. If proper identification of these ANA patterns is done on the basis of the staining pattern of the cell it can definitely help to correlate with a specific type of autoimmune disease.

Thus the aim of this study is to assess the positivity rates of ANA among patients who are suspected or

confirmed of having autoimmune diseases and also to determine the common ANA IFA patterns.

Material and Methods

Study design & population

A retrospective study, which included 3016 sera of patients suspected or confirmed of having AD from November 2021 to October 2022 were considered. These samples were processed at Central Microbiology Lab, SMS Medical College & Hospital.

Method

ANA IFA test was performed with kit (Immunoconcepts made in USA procured from Medsource Ozone Biomedical private limited)

using 1:80 dilutions of the patient serum. When binding of ANA occurs to a particular antigen it leads to emission of fluorescence which is then seen with a fluorescence microscope under 10× and 40× objectives.

The intensity of fluorescence of each sample was then compared with both negative and positive controls which were supplied in the kit. The grouping and then further classification of these ANA patterns were done as per the ICAP (International Congress on Antinuclear Antibody Patterns) nomenclature.

Observation and Results

Out of 3016 samples which were processed 596 (20%) samples came out to be positive. (Fig.1)

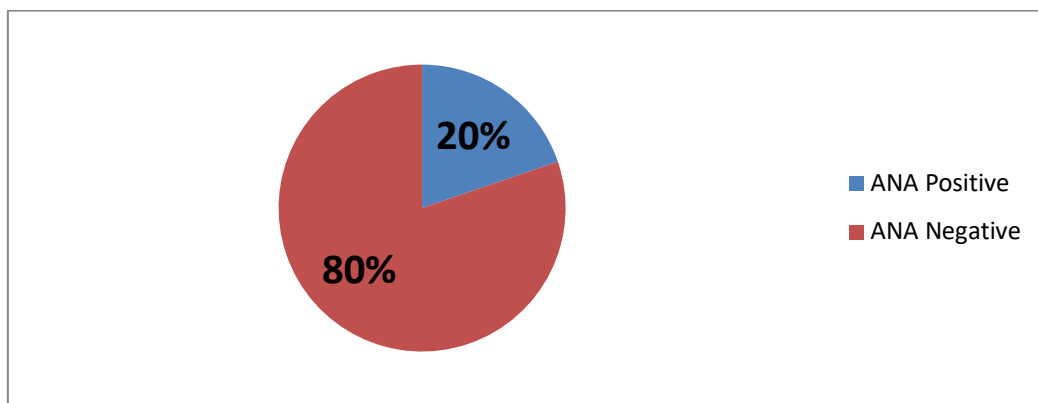


Figure 1: Seroprevalence of ANA among patients suspected for AD

Among the positive patients majority of them were females {461(77.34%)} as compared to males {135(22.65%)}. (Table 1)

Table 1: Sex wise distribution of positive samples

ANA positive males	135(22.65%)
ANA positive females	461(77.35%)
Total	596 (100%)

In our study among those positive majority of them belonged to the age group of 31-50 years (305 {51.17%}). (Fig 2)

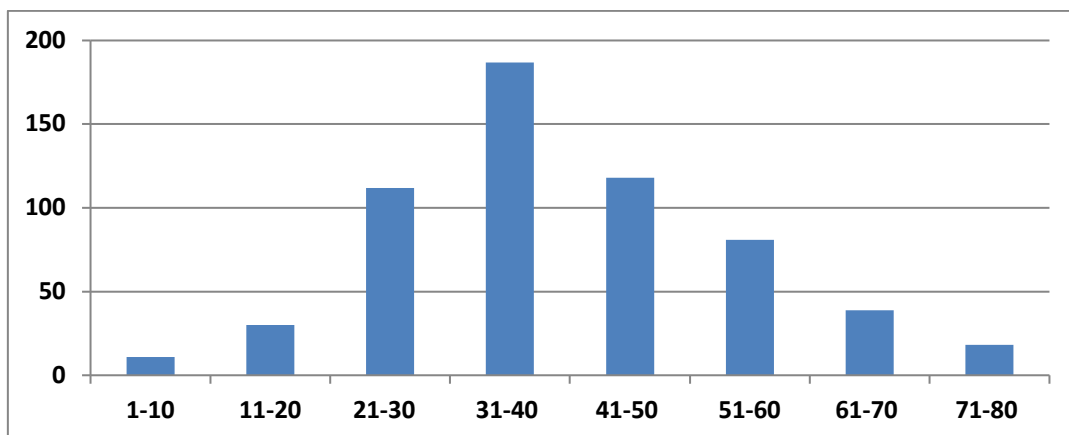


Figure 2: Age wise distribution of samples positive for ANA

Out of the various ANA patterns observed in our study we found that among nuclear component speckled (486{54%}) as the most frequent pattern followed by the homogenous pattern (50{8.3%}). Other patterns observed were nucleolar (19{3.18%}), centromeric(11{1.8%}), SSA-Ro(8{1.34%}) & mixed (22{3.65%}). Among the nuclear negative samples cytoplasmic pattern was seen in 245(10.12%) cases.(Table 2) (Fig. 3A-E)

Table 2: Distribution of different ANA patterns

Component	Type of pattern	N (%)
Nuclear	Speckled	486(81.54)
	Homogenous	50(8.3)
	Nucleolar	19(3.18)
	Centromeric	11(1.8)
	SSA-Ro	8 (1.34)
Cytoplasmic		245 (10.12)
Mixed	Speckled+ Homogenous	22 (3.65)

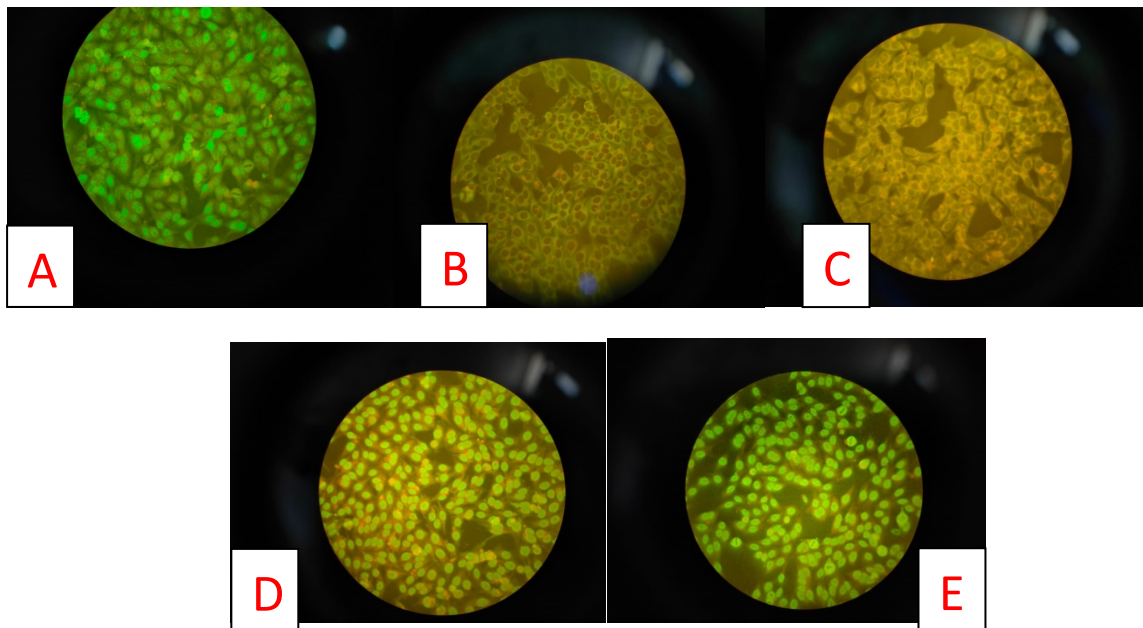


Figure (3A-E): Pictorial representations of various patterns as seen in IIFA

A	SSA Ro
B	Negative cytoplasmic
C	Negative
D	Speckled
E	Homogenous

Discussion

High incidences of autoimmune diseases (ADs) have been reported worldwide. Most of the studies suggest the total incidence of ADs anywhere between 5 to 30%. [9] In our study ANA positivity rate was reported to be 20% (n=596) which was very much similar to studies done by Krzemein et al [1], Maritz et al [10], Prüssmann et al [11]. A higher positivity of ANA was reported by Akmatov, et al. (33%) [3] According to our study the most common age group which is affected is between 31-50 years (n=305) of age which was in concordance with study by Vinita et al [7] (20-40 years), mean age is 43.4 years in a study by Rangnathan et al [6], study by Prishni et al [12] most common age group is 30-39 years however in

study by Krzemein et al [1] 64% patients were over 50 years age.

The positivity was higher in female (n=461{77.34%}) as compared to male patients (22.65%). Similar findings were seen in studies like Vinita et al [7] (70.37% females positive), Reshmy G S et al [8] (73.80%), Krzemein et al [1] (72% females positive), Rangnathan et al [6] and Prishni et al [12]. And one possible explanation for this could be due to hormonal factors as studies suggest that estrogen it affects the immune system and women tend to show a vigorous response because of estrogen making their immune system hyperactive.

Among various IFA patterns in our study most common IFA pattern observed was speckled (48.6{81.54%}) followed by homogenous (50{8.3%}), nucleolar (3.18%), centromeric (1.8%) & SSA-Ro (1.34%) similar findings were reported by Vinita et al [7], Ranganathan et al [6], Krzemein et al [1] and Prishni et al [12]. Though a lot many studies differed from this & a possible reason for this could be the prevalence of one particular type of AD in that area ,thus leading to that particular pattern.

Conclusion

The prevalence of autoimmune diseases is increasing worldwide. An effective method of screening for early diagnosis is much needed as timely intervention of these disorders can result in better outcome for the patient & more such studies are the need of the hour as they help to highlight the epidemiology of these autoimmune diseases which will definitely aid in better diagnosis & early treatment & prevent morbidity

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