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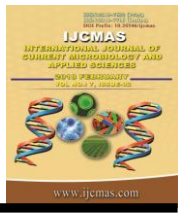

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Screening of Auto Antibodies using Indirect Immunofluorescence in Auto Immune Disease Patients

Ambuja Sekar, G. Sucilathangam* and C. Revathy

Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

The Hallmark of Autoimmune diseases is the pathologic activity of the immune system of an organism directed against its own cells and tissues. The disease is a direct consequence of tissue and/or organ damage as mediated by auto reactive components of the immune system, that is, auto reactive T lymphocytes and/or auto antibodies. For diagnostic purposes, auto antibodies are the most important analytes. Current clinical practice considers Antinuclear Antibody (ANA) testing as a screening test; this has a major impact on laboratory work with a growing volume of analyses that need to be performed rapidly, to maintain good specificity and sensitivity. The present study was undertaken to determine the rate of ANA positivity and their relationship with the various patterns of the positive specimens. In the present study, a total of 558 samples from patients with clinical suspicion of autoimmune diseases were collected during the period of 2016-17 and was tested for ANA by Indirect Immunofluorescence(IIF) with standard procedures. A total of 558 samples were collected and out of which 76(13.6%) were positive for Antinuclear antibodies with the positivity significantly higher in females. Among the positives, most commonly observed pattern was coarse speckled in 27 cases (35.5%). Information about the pattern types and the distribution of the patterns contribute in diagnosis of autoimmune diseases. It is observed that clinical diagnosis has been supported significantly by ANA test according to data of the present study.

Introduction

Autoimmune disease is defined as a condition with tissue destruction or organ malfunction caused by autoimmune mechanisms. It is often more generously interpreted as a disease accompanied by an autoimmune phenomenon, since the direct role of the autoimmune reaction in the disease pathogenesis is not always apparent. Systemic autoimmune are characterized by the presence of non-organ specific auto antibodies that target antigens that are present in virtually any type of cell. Clinically, they are characterized by the systemic involvement of autoimmune tissue destruction in various organs.

One of the common serological hallmarks of autoimmune disorders is the presence of various auto antibodies in the sera of patients affected by these disorders. Auto antibodies to different cellular antigens are the

characteristic finding in several organ specific and non-organ specific autoimmune disorders. (Satoh *et al.*, 2012) Among several different auto antibodies, antinuclear antibodies (ANA) are considered to be the commonest. Antinuclear antibodies (ANA) detection is often needed to aid the diagnosis in several autoimmune disorders. Antinuclear antibodies (ANA) are essential biological markers for the diagnosis (Solomon *et al.*, 2002), classification, and disease activity monitoring (Fritzler, 2008) of systemic autoimmune diseases. ANA have traditionally been carried out by indirect Immunofluorescence (IIF) using HEp2 cells as substrate.

Recently, the American College of Rheumatology (ACR) stated that ANA detection by IIF is still considered the gold standard (Meroni and Schur, 2010). This was primarily based on the high sensitivity of the IIFAssay.Antinuclear antibodies (ANAs) are directed against nuclear antigens and can be grouped into four categories (1) antibodies to DNA, (2) antibodies to histones, (3) antibodies to nonhistone proteins bound to RNA, and (4) antibodies to nucleolar antigens.

The most widely used method for detecting ANAs is indirect Immunofluorescence, which can identify antibodies that bind to a variety of nuclear antigens, including DNA, RNA, and proteins (collectively called generic ANAs).The pattern of nuclear fluorescence suggests the type of antibody present in the patient's serum.

This brief review discusses some methodological aspects of ANA detection and the clinical relevance of the presence of some of the auto antibodies found in the sera of patients with autoimmune disorders. Hence the present study was undertaken to determine the rate of ANA positivity and their relationship with the various patterns of the positive specimens.

Materials and Methods

Serum was collected from the Blood samples sent to the Microbiology laboratory. A total of 558 samples were taken and was tested for ANA by Indirect Immunofluorescence using the Bio systems Immunofluorescence Kit. The procedure was carried out according to the kit manufacturer's instructions.

The serum samples were diluted 1/80. 1 drop each of the Control and Test sera were placed on each slide well, ensuring to cover it completely. Serum anti-nuclear antibodies bind to the corresponding antigens present in the HEp -2 cells coated on the slides.

After an incubation of 30 minutes, the slide was drained and rinsed with phosphate buffered saline. The resulting antigen antibody complexes are detected by means of a fluorescein labeled anti human globulin. After a further incubation for 30 minutes, the slide was rinsed, mounting medium was added and then it was examined under the fluorescent microscope.

The different patterns of fluorescence observed were Homogenous, Peripheral, Speckled, Nucleolar and centromere.

Homogeneous or diffuse nuclear staining usually reflects antibodies to chromatin, histones, and, occasionally, double-stranded DNA.

Rim or peripheral staining patterns are most often indicative of antibodies to double-stranded DNA.

Speckled pattern refers to the presence of uniform or variable-sized speckles. This is one of the most commonly observed patterns of fluorescence and therefore the least specific. It reflects the presence of antibodies to non-DNA nuclear constituents.

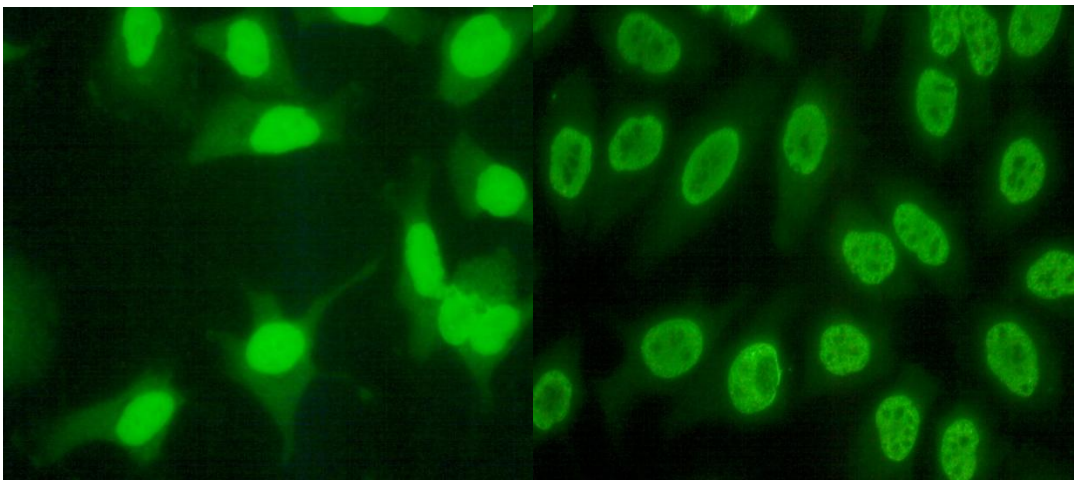
Table.1 Age wise distribution among positive cases

Sl no	Age	Females	Males
1	11 - 20	11	4
2	21 - 30	18	2
3	31 - 40	23	3
4	41 - 50	4	2
5	51 - 60	5	2
6	61 - 70	1	0
7	71 - 80	1	0
	Total	63	13

Table.2 Distribution of Immunofluorescence patterns of observed in samples

Sl no	Pattern	Females	Males
1	Coarse speckled	22	5
2	Cytoplasmic	18	3
3	Homogenous	9	3
4	Membranous	8	1
5	Centromere	4	1
6	Nucleolar	2	-

Fig.1 Immunofluorescence patterns (Homogenous pattern and Coarse speckled pattern)



Nucleolar pattern refers to the presence of a few discrete spots of fluorescence within the nucleus and represents antibodies to RNA. This pattern is reported most often in patients with systemic sclerosis.

Results and Discussion

A total of 558 samples were tested for a period of one year between 2016 and 2017. Out of which 76 (13.6%) were positive for

ANA by Indirect Immunofluorescence. Out of the 76 positives, 63 were females and 13 were males. Age wise distribution among Positive cases was depicted in Table 1. The most commonly involved age group belongs to 30-39 years.

The Indirect Immunofluorescence shows different ANA patterns. Table 2 shows the different patterns observed, of which Coarse speckled was the predominant pattern found in 27 cases (35.5%), followed by cytoplasmic seen in 21 cases (27.6%), while homogenous was present in 12 cases (15.8%), Membranous in 9 cases (11.8%), centromere in 5 cases (6.6%) and nuclear pattern seen rarely in 2 cases (2.6%) (Figure 1).

ANA- IIF plays an important role in diagnosis of autoimmune diseases. Determination of ANA patterns using Indirect Immunofluorescence (IIF) is useful in differential diagnosis. ANA tests are easy to perform and have low cost, ANA tests have remained important because particularly some of the patterns are extremely helpful in the diagnosis and in some cases the clinician needs to be supported by the IIF.

In the present study, ANA positivity rate, distribution of ANA patterns according to sex and age were evaluated. ANA positives were more among the females (11.3%) than the males. (2.3%) This correlates with the findings of Hayashi *et al.,* who reported that of the 111 patients with SLE, 104 were women and were men, and the median age was 35 years (Hayashi *et al.,* 2001). In the present study, most of the ANA positives belonged to the age group of 31 to 40 years.

The most frequently reported pattern was coarse speckled (Granular) found in 27 cases (35.5%), followed by cytoplasmic seen in 21 cases (27.6%), while homogenous was present in 12 cases (15.8%), Membranous in 9 cases (11.8%), centromere in 5 cases (6.6%) and nuclear pattern seen rarely in 2 cases (2.6%). It was reported that ANA positivity could be observed in 95% in mixed connective tissue disorders. Sebastian *et al.,* have observed homogenous pattern in 45.5%, speckled pattern in 35.6% of the ANA positive samples. (Sebastian *et al.,* 2010) Sunitha *et al.,* reported that a cytoplasmic granular pattern in 37 % of the samples and a homogenous pattern in 23 % of the samples. (Sunitha *et al.,* 2012)

In the current scenario of diagnosis of autoimmune diseases based on ANA serology testing for antigen-specific auto antibodies can enable stratification of patients into particular autoimmune diseases (Yazdany *et al.,* 2013). However, in situations where there is minimal or even no suggestion of an immune mediated disease other than vague symptoms, screening for ANA could be most impactful (Fritzler, 2016). The availability to primary care physicians of a convenient ANA testing platform could enhance the goal of improving patient outcomes and reducing health care cost.

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