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Evaluation of Antinuclear Antibody in Connective Tissue Disorders

Biochemistry

KEYWORDS : Connective tissue disorders, ANA, ELISA method for ANA

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ABSTRACT

Connective tissue disorders (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. Although lower amounts of these antibodies can be seen in the normal population as well, a spurt in titers is seen in patients of CTD. Not only are these antibodies involved in the disease pathogenesis, but they also constitute the basis for diagnosis and treatment of CTD. In the present cross-sectional study, 100 clinically suggestive Patients of connective tissue disorders and 100 normal patients were selected. All samples were analyzed for ANA by ELISA method. Sensitivity and specificity of ANA test by ELISA is 87% and 91% respectively. Thus to conclude, it appears that the ELISA assay for ANA is suitable as a preliminary investigation for the diagnosis of Connective tissue disorders. Being technically simpler, less subjective, and faster it could be particularly useful in laboratories screening large numbers of samples.

Introduction

Connective tissue disorders are defined as Heterogeneous disorders that share certain common features, including inflammation of skin, joints, and other structures rich in connective tissue; as well as altered patterns of immunoregulation, including production of autoantibodies and abnormalities of cell-mediated immunity.[1] ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells.[2] Their detection with high sensitivity and specificity is of utmost importance element for diagnosis of CTD.

Presently the ANA have been categorized in to 2 main groups:

1) Autoantibodies to DNA and Histones[3-7]
2) Autoantibodies to extractable nuclear antigens (ENA)[8-16] (e.g. Autoantibody to sm, RNP, SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1)

Table 1: Sensitivity and specificity of ANA and its clinically important subtypes

Autoantibodies	Associated CTD	Sensitivity	Specificity
ANA	SLE	93	57
	Sjogren's syndrome	48	52
	Systemic Sclerosis	85	54
	Polymyositis/dermatomyositis	61	63
	Raynaud's phenomenon	64	41

In recent years, the ANA test has experienced many changes that have increased its specificity and sensitivity. Today, the ANA test is considered a mainstay for helping to diagnose several different autoimmune disorders. Therefore, a positive ANA result in conjunction with clinical findings is diagnostic which is frequently asked by the clinician in case of suspected CTD. Considering these facts we evaluated the utility of ELISA based ANA screening method & its application in day to day laboratory practice.

Materials & Methods

In the present cross-sectional study, 100 clinically suggestive Patients of connective tissue disorders and 100 normal patients were selected from Sheth V S General Hospital. All patients are primarily evaluated by clinicians for clinical examination and other relevant investigation for diagnosis of connective tissue disorders.

Study Groups

Group 1–Patients clinically suggestive of Connective Tissue Disorders (n= 100)

Group 2 – Normal Subjects (n= 100)

Inclusion Criteria for Group 1

- Patients having clinical signs & symptoms of connective tissue disorders like rash, arthritis, fatigue, vasculitis, alopecia, oral ulcers, photosensitivity etc.
- Agree to comply with the study

Inclusion Criteria for Group 2

- Clinically apparently healthy persons having no signs & symptoms of Connective tissue disorders
- Age & Sex matched Persons
- Agree to comply with the study

Exclusion criteria for both Groups

- Known case of connective tissue disorder
- Immuno-Deficient patients
- Patients on immune Therapy
- Critically ill patients

Sample Analysis

All samples were subjected to analysis for measurement for ANA immediately. The measurement of ANA was done by ELISA Technique by Dr.Fenning Ready to use kits for ANA. Results were assured as per standard quality control regime. The study was approved by the Institutional review committee.[17]

Statistical Analysis

Statistical analysis in terms of average age for male & female, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value were done with appropriate statistical calculation.

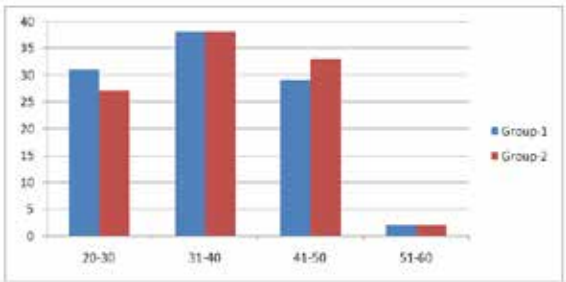
Results

In Study Group-1, Out of total 100 patients, 21 were males and 79 males and average age was 34.33. While, In Control Group-2, Out of total 100 patients, 35 were males and 65 were females.

Table 2: Showing total no of male and female & Average Age in Group-1 & Group-2

	Group-1 (Study Group)	Group-2 (Control Group)
Male	21	35
Female	79	65
Age	34.33	35.66

Graph 1: Age (Years) wise distribution of Group-1 & Group-2



In Study Group-1 out 100 individuals, 87 (87%) were positive for ANA by ELISA method, While in Control Group-2 out of 100 individuals, 9 were positive for ANA by ELISA method.

Graph 2: Comparison of positive & Negative patients in both groups

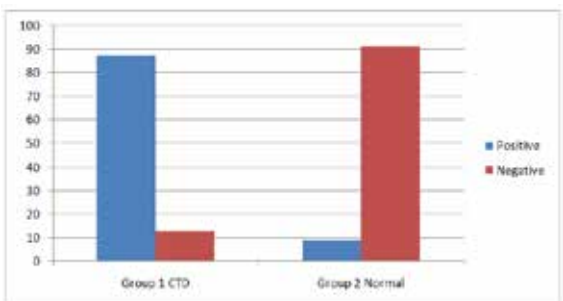


Table 3: Showing True positive, True negative, False positive, False Negative Patients

True Positive(a)	87
True Negative(d)	91
False Positive(c)	9
False Negative(b)	13

Table 4: Showing Sensitivity, Specificity, Positive Predictive value, Negative Predictive value, Disease Prevalence

Sensitivity=a/a+b	87%
Specificity=d/c+d	91%
Positive Predictive Value=a/a+c	90.62%
Negative Predictive Value=d/b+d	87.50%

From table 4, it can be said that sensitivity and specificity of ANA test for diagnosis of Connective tissue disorders is 87% and 91% respectively.

Discussion

Anti-nuclear antibody (ANA) testing is widely used as a screening test in connective tissue disorders. Although the ANA is a commonly ordered study, it is a notoriously misunderstood test. The major use of ANA testing is as a diagnostic tool. If a diagnosis has already been confidently established without ANA testing, the test generally does not need to be performed. It is notable that 57% of ANA tests ordered by clinicians in one prospective study were in patients whose diagnoses were already established.18 However, it has been reported earlier that positive ANA results are found in a significant proportion of the elderly population and sensitivity of ANA testing varies widely from one clinical disease to another. For example, ANA testing has been reported to be positive in > 95% of patients with SLE but in only 10 to 50% of patients with dermatomyositis and polymyositis.

It is well established that the results of autoantibody tests including ANA have only a supportive value and that the diagnosis of most of the connective tissue disorders is based primarily on the presence of a set of defined clinical manifestations.[17]

A positive test for ANA does not, by itself, indicate the presence of connective tissue disorder. It is well established that the results of autoantibody tests including ANA have only a supportive value and that the diagnosis of Connective tissue disorder is based primarily on the presence of a set of defined clinical manifestations.[17]

Some important aspects to be kept in mind while evaluating measuring & evaluating ANA

- ANA testing is not recommended to evaluate fatigue, back pain or other musculoskeletal pain unless accompanied by one or more of the clinical features in favor of a CTD.
- ANA testing should usually be ordered only once.
- Positive ANA tests do not need to be repeated.
- Negative tests need to be repeated only if there is a strong suspicion of an evolving CTD or a change in the patient's illness suggesting the diagnosis should be revised.
- A positive ANA test is important only in conjunction with clinical evaluation and in the absence of symptoms and signs of a CTD; a positive ANA test only confounds the diagnosis. A positive ANA test can also be seen in healthy individuals, particularly the elderly or in a wide range of diseases other than CTD, where it has no diagnostic or prognostic value.

From this study, we found that connective tissue disorders are more common in 30-50 years of age group especially in females. Sensitivity and specificity of ANA measurement by ELISA method in the present study is 87% and 91% respectively. Given the significant rate of positive ANA results in the general population, a positive ANA test should not be over-interpreted.

Conclusion

To Conclude, Connective tissue disorders are more common in 30-50 years of age group especially in females compared to males. Sensitivity and Specificity of ANA test by ELISA method in present study was 87% and 91% respectively. It appears that the ELISA assay for ANA is suitable as a preliminary investigation for the diagnosis of Connective tissue disorders. Being technically simpler, less subjective, and faster it could be particularly useful in laboratories screening large numbers of samples as many amongst these might be ANA negative. It is also more economical than the IF-ANA and this fact has important bearing in our country where IF-ANA may often be unaffordable or not feasible.

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