Detection Of Antinuclear Antibodies: Comparative Evaluation Of Enzyme Immunoassay And Indirect Immunofluroscence Methods

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Abstract:

The suspicion of autoimmune disease primarily starts with clinical symptoms. Serological tests for Antinuclear Antibodies (ANA) have an important role towards the diagnosis of various autoimmune diseases.

Objective-To compare ELISA and IIF on HEp-2 cell methods for detecting ANA. A total of 122 serum sample obtained from patients with established or suspected autoimmune diseases and 61 samples for ANA test, 24 for anti dsDNA test and 37 for antiCCP test using both IIF and ELISA methods. Sensitivity of ANA was found to be 73 % and specificity was found to be 47%. Based on the results ANA was found to detect 73% of the immune-fluorescence test positive cases correctly and able to detect 47% of the immune-fluorescence test negative cases correctly. Specificity of ANA in the current study is found to be quite low, although sensitivity was optimum.

Sensitivity of Anti CCP was found to be 39 % and specificity was found to be 64%. Based on the results Anti CCP was found to detect 39% of the immune-fluorescence test positive cases correctly and able to detect 64% of the immune-fluorescence test negative cases correctly.

It was concluded that IIF on HEp-2 cell was more sensitive than ELISA in detecting the total ANA . ELISA prescreening combined with IIF can obtain the information of the nuclear pattern and allow the observation. The combination of two testing methods can greatly enhance the accuracy of the results.

Key words: Discolored broken matured permanent teeth, non-vitality, apical, radiolucency, full veneer crown.

Introduction

The measurement of autoantibodies against antigens of the nucleus (antinuclear antibodies (ANA) is commonly used for screening, diagnosis, and monitoring of connective tissue diseases (CTD) such as systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), mixed connective tissue disease (MCTD), Sjogren syndrome (SS), and polymyositis (PM). The preferred technique is indirect immunofluorescence (IIF) on HEp-2, a human epithelial cell line, as an antigen source [1,2]

More recently, enzyme immunoassays (EIA) have been introduced for the detection and measurement of ANA. They differ mainly by the antigen composition used in each well: while screening tests use whole HEp-2 nuclei, an extract thereof, or a mixture of defined nuclear antigens, diagnostic tests use a single defined antigen, allowing the qualitative assessment of four to six different antibodies, i.e., an antibody profile, in one run [3].

Compared to IIF, the EIA technique is objective, is less labor-intensive, and has the potential for automation. At the same time, however, it is more expensive. It provides results in optical densities (ODs) rather than titers and gives the antibody specificity rather than the ANA pattern, i.e., it has an impact both on the logistics of clinical laboratories performing the ANA test and on the thinking of the clinician ordering it. No doubt, this technique has been put on the market in the hope that it will supplement the existing IIF technique or even replace it[4].

Our study is, based on a fairly large number of consecutively collected, clinically defined sera, and the

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data were obtained at a routine laboratory. In addition, we provide an extensive validation of the IIF on HEp-2 cell technique as such, with one of the laboratories comparing HEp-2 and against an ANA screen EIA[5].

Materials and Methods:

This experimental study was conducted on a total of 122 subjects. Laboratory work was carried out in the Department of Microbiology & Immunology, Rheumatology Center and SLE clinic of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh during the period of July 2008 to June 2009.

All subjects were interviewed and findings recorded on a pre designed data sheet irrespective of age and sex. Out of 122 patients - 61 were tested for ANA, 24 for Anti ds DNA test and 37 were tested for Anti CCP respectively sample collection and storage.

Approximately 5 ml blood samples were collected by venepuncture and sera was separated as soon as possible, aliquoted and stored at -20 ^{0} C, repeated thawing and freezing was avoided.

Laboratory method by-

A) Detection of antinuclear antibody by indirect immunofluorescence method on HEp-2 cell line .

B) Detection of Antinuclear Antibodies (ANA) by ELISA method .

C) Detection of Anti ds DNA by ELISA method.

D) Detection of Anti CCP by ELISA method.

Result

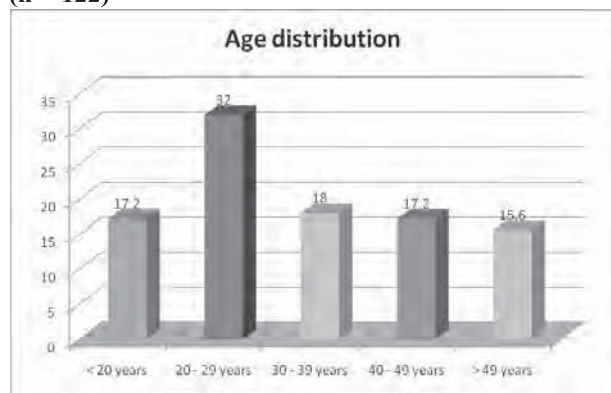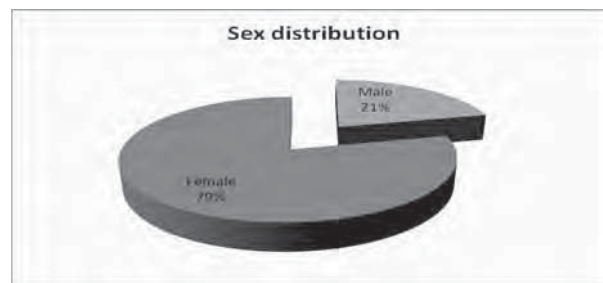**Figure 1: Distribution of the study population by age (n = 122)**

Figure 1 shows the distribution of the respondents among the study participants highest percentage were in between 20-29 years age group that was 32%.

Figure 2: Distribution of the study population by Sex (n=122)

Pie chart shows the distribution of the study participants by sex. Among the participants 21.3% were male and 78.7% were female.

Table 1: Results of ELISA done for Anti CCP, Anti dsDNA and ANA test in study population(n=122)

Table 1.1 ELISA done for ANA of study groups (n=61)

ANA	Frequency	Percentage
ANA+ve	38	62.3
ANA-ve	23	37.7
Total	61	100.0

Antinuclear Test for anti nuclear antibody test was done in 61 patients among them 62.3% were found to be positive (ANA +ve) and 37.7% were found to be negative (ANA).

Table 1.2: ELISA done for AntidsDNA of study groups (n=24)

AntidsDNA	Frequency	Percentage
AntidsDNA+ve	15	62.5
AntidsDNA-ve	9	37.5
Total	24	100.0

Anti ds DNA Frequency Percent Antids DNA+ve 15 62.5 Anti ds DNA-ve 9 37.5 Total 24 100.0

Test Anti ds DNA was done in 24 patients among them 62.5% were found to be positive (Anti ds DNA +ve) and 37.5% were found to be negative (Anti ds DNA -ve).

Table 1.3: ELISA-Anti CCP done in study group (n=37).

ELISA	Frequency	Percentage
Anti CCP + ve	14	37.8
Anti CCP - ve	23	62.2
Total	37	100

ELISA test was done in 37 patients, among them 37.8% were found to be Anti CCP positive + ve and 62.2% were found to be Anti CCP – ve.

Figure 3: Accuracy of ANA in comparison to Immuno-fluorescence in terms of sensitivity, specificity, PPV, NPV and Accuracy
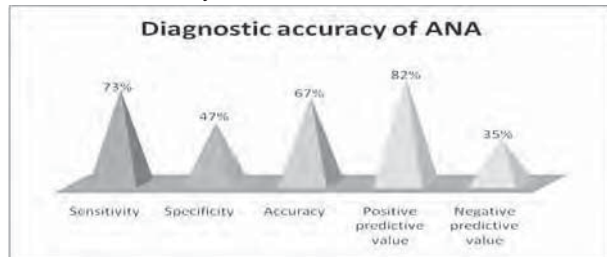
Diagnostic preciseness of ANA results were assesed based on immunoflouresence test. Sensitivity of ANA was found to be 73 % and specificity was found to be 47%. Based on the results ANA was found to detect 73% of the immune-fluorescence test positive cases correctly and able to detect 47% of the immune-fluorescence test negative cases correctly. Specificity of ANA in the current study was found to be quite low,

Discussion

Test for antinuclear antibody (ANA) in serum is now an accepted part of routine diagnostic evaluation in patients with connective tissue disease. Detection of ANA by indirect immunofluorescence(IIF) method is highly sensitive for CTD. In this study we calculated both of these performance characteristics for a commercial ELISA & IIF method for ANA. The result presented in this study shows that maximum CTD patients were in 20-29 years of age group followed by 30- 39 years group and among CTD patients 21.3% were male and 78.7% were female.

Several studies revealed that CTD condition was the increased susceptibility of the female sex. Women are at least tenfold more likely to develop SLE [6].

In this study, ELISA and IIF -test were done in 122 patients. Among them ANA was done in 61 patients , Anti ds DNA done in 24 patients and Anti-CCP test was done in 37 patients.

Antinuclear antibody test done by ELISA in 61 patients, among them 62.3% were found to be positive ANA and 37.7% were found to be negative ANA.

Test Anti dsDNA was done by ELISA in 24 patients, among them 62.5% were found to be positive AntidsDNA and 37.5% were found to be negative Anti dsDNA.

Among 37 patients of Anti-CCP, 37.8% were found to be Anti CCP positive and 62.2% were found to be Anti CCP negative. ANA has been reported positive and in high titres in almost all cases of SLE [7,8,9,10,11,12].

Immuno-fluorescence test was done in 122 subjects. The test is considered as gold standard test to assess the accuracy of

,ANA Anti dsDNA and Anti-CCP test. Diagnostic preciseness of ANA results were assesed based on immunoflouresence test. Sensitivity of ANA was found to be 73 % and specificity was found to be 47%. Based on the results ANA is found to detect 73% of the immune-fluorescence test positive cases correctly and able to detect 47% of the immune-fluorescence test negative cases correctly.

Another study shows the positivity rate of ANA detected by IIF and Anti-CCP was significantly different (87.8% and 73.17%, respectively, P<0.01), but the positivity rate of anti-dsDNA was similar between IIF and ELISA (77.19% and 71.93%, respectively, P>0.05). The percent agreement between the two testing methods with different cutoff values of ANA and anti-dsDNA showed significant differences (P<0.01) [13,14].

IIF and ELISA both are important but IIF has more sensitivity than ELISA but yet not IIF is not commonly practiced in our country. So this study results would be helpful to our immunological and microbiological field for screening of CTD by indirect Immunofluorescence technique.

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