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Original Article

Evaluation of indirect immunofluroscence on HEP-2 cell and enzyme immunoassay methods for detection on antinuclear antibodies

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Abstract

The suspicion of autoimmune disease primarily starts with clinical symptoms. The ELISA and IIF (indirect immunofluorescence) on HEp-2 cell methods are comparable for detecting ANA in patient with auto immune diseases (AID). 102 patients attending Rheumatology out patient department as new or old cases with provisional diagnosis of SLE, RA and other Connective Tissue disorder (CTD) screened for specific autoantibody by ELISA (anti CCP, ANA and anti dsDNA) were subjected to indirect immunofluorescence on HEp-2 cell line. Diagnostic accuracy based on ELISA and indirect immunoflurescence on HEp-2 cell line showed the results of sensitivity, specificity, PPV, NPV, accuracy of ANA were found to be 78.9%, 33.3%, 78.9%, 33.3%. 68% respectively. Sensitivity, specificity, PPV, NPV and the accuracy of anti dsDNA was 80%, 40%, 80%.40% and 70% respectively. Sensitivity, specificity, PPV, NPV, and the accuracy of anti -cyclic citrullinated peptied (CCP) was found to be 64.3%, 72.2%, 64.3%, 72.2% and 68.8% respectively. The results indicates there is a need for screening all suspected autoimmune patient by indirect immunofluorescence on HEp-2 cell line before going to specific test .

Key words: HEp-2, Antinuclear antibodies

Introduction:

Antinuclear antibodies (ANA) are antibodies of different specificity directed against antigens of the cell nucleus and are a group of antibodies directed against various nuclear and some cytoplasmic antigens. Determining the presence and specificity of antinuclear antigens (ANA) is a challenge to a laboratory involved in the diagnosis of connective tissue disease (CTD). The immunofluorescent technique (IIF), once considered the gold standard is gradually displaced by ELISA[1,2].

Detection of autoimmune disease relies solely on serological methods (detection of antibody) as because other options like biochemical or other parameter is not possible. Consequently serological tests for ANA detection is a mandatory step towards the diagnosis of various autoimmune connective tissue disorder like (MCTD) and Sjogrens syndrome[3,4,5]. There are various methods for detection of ANA. The most commonly used methods are indirect immunofluorescence method and enzyme immuno assay [6].

ANA can be divided into -extractable nuclear antigen (ENA), non extractable nuclear antigen, and cytoplasmatically located antigen. ANA are usually detected by indirect immunofluroscence on HEp2 cell[7]. Indirect IF on HEp2 cell which may also help to identify the reaction pattern of the ANA specific for a particular CTD[8]. Using single method (EIA) for such a large range of diseases and patients makes sensitivity and specificity of our test questionable. Since IIF test has been marked as a screening test by several workers/ studies[6].

Thus an attempt has been made in our study to see the variation in the level of sensitivity and specificity of EIA test use alone and with IIF on our patients, seeking diagnosis or treatment for autoimmune diseases.

Materials And Methods:

TThis experimental study was conducted on a total 102 subjects. Laboratory works were carried out in the Department of Microbiology & Immunology, Rheumatology

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Center and SLE clinic of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh in 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 102 patients - 50 patients were selected for ANA test, 20 patients were for anti ds DNA test and 32 patients were for anti CCP test.

Sample collection and storage

Approximately 5 ml blood sample was collected by venepuncture and sera was separated as soon as possible, aliquoted and stored at -20°C. Repeated thawing and freezing was avoided.

Laboratory method

Detection of antinuclear antibody by indirect immunofluorescence method on HEp-2 cell line; Detection of antinuclear antibodies (ANA) by ELISA method; Detection of anti dsDNA by ELISA method and detection of anti CCP by ELISA method was done with standard procedure[9].

Results:

Table -I: Distribution of the study subjects by age (n= 102)

Age group in Years	Number	Percentage (%)
< 20 years	17	16.6
20 - 29 years	33	32.3
30 - 39 years	20	19.6
40 - 49 years	17	16.6
> 49 years	15	14.7
Total	102	100

Most of the patients were 20-50 years age group, of them (32.3% of the patients were in 20-29 years age followed by 19.6% in 30-39 years age group and 16.6% in 40-49 years age.

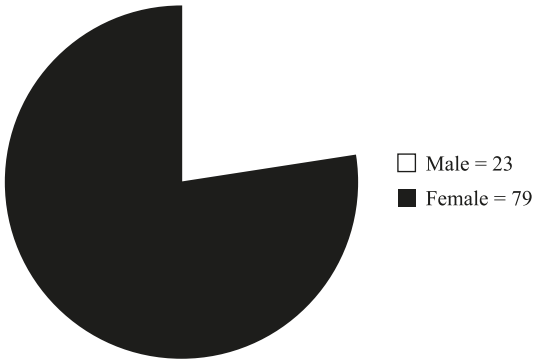

Figure -1. Distribution of the study subjects by sex (n 102)

Among the participants 23(22.5%) were male and 79(77.5%) were female. Anti dsDNA were found positive in 75% cases ANA were found positive in 76% cases and Anti CCP were found positive in 43.75% cases. (Table II).

Table-II: Results of ELISA test for anti CCP, anti dsDNA and ANA test on study subjects (n= 102)

ELISA Result	Positive n(%)	Negative n(%)	Total n(%)
ANA	38 (76.00)	12 (24.00)	50 (100)
anti ds DNA	15 (75.00)	5 (25.00)	20 (100)
anti CCP	14 (43.75)	18 (56.25)	32 (100)

Table-III: Results of ANA test by ELISA and its correlation with Immune fluorescence (n=50)

ANA by ELISA	Immunofluorescence positive	IF Negative	Total
ANA positive	30 (78.90)	8 (21.10)	38 (100)
ANA Negative	8 (66.70)	4 (33.30)	12 (100)
Total	38 (76.00)	12 (24.00)	50 (100)

Chi-Square= 1.54 df = 1 P = .076[NS]

Among the 38 study subjects(76.0%) were ANA positive by immuno-fluorescence. Among the 12 study subjects 8(66.7%) were ANA positive by immuno-fluorescence. No statistically significant difference was found between immuno-fluorescence test and ANA test (P>.05). (Table III)

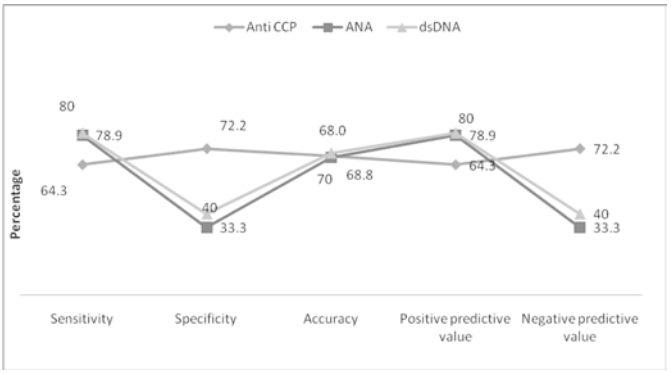

Figure II: Comparison of diagnostic performance of anti-CCP, ANA and anti dsDNA (n=102)

AntiCCP by ELISA is found to have almost similar sensitivity specificty and predictive values. Both ANA and anti dsDNA shows similar pattern of accuracy. Both the tests were found to have higher sensitivity and positive predictive values. These two test shows lower specicity and negative predictive value. The overall accuracy were (anti-CCP was 68.8%, ANA was 68.0% and anti dsDNA was 70.0%) respectively.(Figure II)

Table-IV: Anti nuclear antibody fluorescence pattern in relation to fluorescence intensity (n=102)

Fluorescence Pattern	Number	Percentage
Nuclear Homogenous	45	44.1
Coarse speckle	8	7.8
Fine speckle	8	7.8
Nuclear matrix	6	5.8
Fibrous cytoplasm	5	4.9
Nuclear pore	3	2.9
Nuclear membranous	5	4.9
Cytoplasmic filaments	3	2.9
Others	19	18.6
Total	102	100%

Note; Others- Patterns not identifide.

This table (IV) shows the distribution of fluorescence pattern in relation to fluorescence intensity in this cases (n=102). Most prevalent (44.1%) fluorescence pattern are Nuclear Homogenous pattern followed by coarse speckle 7.8%), Fine speckle (7.8%), Nuclear matrix (5.8%), Fibrous cytoplasmic (4.9.0%), Nuclear pore pattern (2.9%), Nuclear membranous pattern (4.9%). cytoplasmic filaments (2.9%) and others (18.6%) are identified.

Discussion:

Detection of ANA by indirect immunofluorescence on HEp-2 cell method is highly sensitive for CTD[7].Current method of choice for ANA detection is IIF[10]. ELISA is commonly practicing as a method of choice for specific antibodies and more recently, autoantibody detection is a useful method, the test for ANA must be sensitive and capable of detecting a wide variety of specific autoantibodies. The IIF method performed on substrate slides of cultured epithelial cells (HEp-2 cells) has met both of these criteria for clinical usefulness [10].

The result of this study shows that maximum CTD patients were in 20-29 years of age group followed by 30- 39 years group. Among CTD patients, 22.5% were male and 77.5% were female. Several studies shows female are more susceptible than male for acquiring CTD [10].Women are at least tenfold more likely to develop SLE, efforts to understand this female tendency to develop autoimmune diseases have not been entirely successful, but hormonal influences play a major role, and endocrinologic abnormalities have been described [10].

In this study, ELISA and IFA-test were done on 102 patients for case group and 20 for healthy group. Among 32 patients who underwent anti-CCP test 14(43.75%), were found to be positive and 18 (56.25%) were found to be anti CCP negative. Test for anti dsDNA by ELISA on 20 patients showed that 15 (75.0%) were positive and 5 (25.0%) were negative for anti dsDNA.

ELISA test for antinuclear antibody (ANA) on 50 patients showed that 38 (76.0%) were positive, while 12 (24.0%) were negative for ANA. Among the CTD, ANA test by EIA or IIF has been successfully used to detect SLE cases in a wide range of titer[11-16]. Another study reveals a negative ANA and antidsDNA by EISA rules out the possibility of SLE in such cases. However, many investigators found no clinically significant titer for any connective tissue diseases. In western studies a high titer of ANA (1;80-1;320) was a characteristic feature of SLE[17] .

As SLE is a disease of young women our results showed majority of the cases are between (20-29) years age group has similarity with other studies[17]. This study shows results of the ANA test by ELISA and its correlation with IIF out of 38 study subjects 76.0% were ANA positive by immunofluorescence. IIF has been repeatedly marked as more sensitive test in most literatures[18,19]. On the other hand, out of 12 study subjects 8(66.7%) were positive by IIF. Patients may have variety of autoantibodies other than ENA. This indicates the ability of the IIF to detect wide range of autoantibody which is not possible with ELISA method. No statistically significant difference was found between immunofluorescence test and ANA test (p>.05).

The immuno-fluorescence test is considered as gold standard for detection of autoantibodies, assess the diagnostic preciseness of anti dsDNA ANA and antiCCP results in this study was based on ELISA and IIF on HEp-2 cell line . Sensitivity of anti dsDNA was 80.0% and specificity was 40.0%, positive 'predictive value and negative predictive value was 80% and 40% respectively, and the accuracy was 70.0%. Sensitivity of ANA was 78.9 %, specificity was 33.3%, positive predictive value 78.9%, negative predictive value 33.3% and the accuracy was 68.0% .

This study shows in table IV that, the most prevalent (44.1%) fluorescence pattern are nuclear Homogenous pattern followed by coarse speckle (7.8%), fine speckle (7.8%), nuclear matrix (5.8%), fibrous cytoplasmic (4.9.0%), nuclear pore pattern (2.9%), nuclear membranous pattern (4.9%). cytoplasmic filaments (2.9%) and others (18.6%) are identified.

It can be concluded that both ELISA and IIF on HEp-2 cell has an important role in CTD diagnosis. IIF is a promising viable option and should certainly have a wider acceptance to be performed for a specific diagnosis. So this study is expected to be of great importance in guiding patient to pass through an appropriate channel for a definite diagnosis of CTD.

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