Original Article

The Evaluation of ANA and dsDNA results in patient with suspected autoimmune disease

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Abstract. Antinuclear antibodies (ANA) and double-stranded DNA (dsDNA) antibodies are widely used for diagnosis of autoimmune disease. The aim of this study was to investigate the prevalence of ANA and anti-dsDNA in patient with suspected autoimmune disease.

Serum samples were obtained from different clinics of Sakarya Educational and Research Hospital. Each of these serum samples was tested for the presence of ANA and anti-dsDNA by ELISA technique. These tests were performed by commercial kits according to the manufacturer's instructions. ANA and anti-dsDNA results were classified as positive or negative for each patient. Borderline results were arbitrarily classified as positive.

In this study, ANA and anti-dsDNA were found positive 2.96% (58/1975) and 4.52% (29/642) respectively. There is no statistical significant correlation between ANA and anti-dsDNA positivity. Only two patient both ANA and anti-dsDNA were found positivity by ELISA kits. Agreement between assays is generally marginal.

ELISA technique seems to be less sensitive than fluorescent tests for ANA with fewest positivity rates. These results may be due to a number of factors which may contribute to the variability on ELISA. Finally, each of the autoantibody assays provides different criteria for diagnosis, but ANA screen test should be followed up with fluorescent tests to provide proper diagnostic information.

Key words: Autoimmunity, ANA, anti-dsDNA

1. Introduction

Anti-nuclear antibodies (ANA) are immunoglobulin directed against autologous cell nuclear and cytoplasmic components (1-3). The occurrence of different ANA is associated with autoimmune disease and with differences in disease severity including extent of skin involvement, internal organ manifestation and prognosis (2). Researchers have been performing steady efforts to develop tests for detecting ANA and disease-specific auto antibodies to nuclear antigens for the diagnosis, prognostic assessment, and monitoring of patients with systemic autoimmune diseases (4).

Nowadays, measurement of ANA has been widely used to provide supporting evidence of a diagnosis of autoimmune disease such as systemic lupus erythematosus (SLE), Sjögren etc. (5). SLE is a multisystem disorder that is considered as a prototype immune complex (IC)-mediated disease (6). This autoimmune disease related to central or peripheral nervous system; about 17% to 75% of patients respectively (7). Additionally, levels of antibodies against dsDNA were shown covary with SLE disease activity (8). The aim of this study was to investigate the prevalence of ANA and anti-dsDNA in patient with suspected autoimmune disease.

2. Materials and methods

Serum samples were obtained from Sakarya Educational and Research Hospital. Each of these serum samples was tested for the presence of ANA (Aeskulisa ANA, Aesku Diagnostics, Germany) and anti-dsDNA (Aeskulisa dsDNA check, Aesku Diagnostics, Germany) by ELISA method. These tests were performed by commercial kits according to the manufacturer's instructions. First of all, results were classified as ANA positive or negative according to the definitions contained within the packages for each kit. Subsequently, anti-dsDNA results were classified as positive or negative for each patient. Borderline results were arbitrarily classified as positive. For quantitative interpretation establish the standard curve by plotting the optical density

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Table 1. Distribution of ANA test results in study period

	Positive		Negative		p
	Male	Female	Male	Female	
	n (%)	n (%)	n (%)	n (%)	
2009 (n=556)	2(0.4)	18(3.2)	201(36.2)	336(60.4)	
2010 (n=903)	1(0.1)	21(2.3)	211(23.4)	670(74.2)	>0.05
2011 (n=516)	2(0.4)	14(2.7)	141(27.3)	359(69.6)	

Table 2. Distribution of anti-dsDNA test results in study period

	Positive		Negative		p
	Male	Female	Male	Female	
	n (%)	n (%)	n (%)	n (%)	
2009 (n=167)	0(0)	1(0.6)	68(40.7)	115(68.9)	
2010 (n=179)	1(0.6)	5(2.8)	56(31.3)	120(67.0)	<0.05
2011 (n=296)	8(2.7)	14(4.7)	76(25.7)	199(67.2)	

(OD) of each calibrator with respect to the corresponding concentration values in U/mL.

For statistical analyses, manufacturers suggested cut-off were applied to create positive and negative values from the continuous original observations. Positivity rates, specificities and Spearman correlation coefficient between assays were calculated as indicated using SAS software, Version 9.2 of the SAS system for Windows. In statistical analyzes, p-value <0.05 was considered as significant.

3. Results

In this study, we evaluated 1975 ANA and 642 anti-dsDNA results that examined during 3 years period retrospectively. Studied samples were sent from different clinics in Sakarya Educational and Research Hospital between 2009 and 2011. A total of 58 (2.96%) sera were found ANA positive. ANA positivity rates in woman were calculated as 5.08%, 3.04% and 3.75% for 2009, 2010 and 2011 respectively. There were no statistically significant differences for ANA positivity for both males and females in study period. Other results were summarized in Table 1.

Twenty nine (4.52%) patients were found positive for anti-dsDNA (Table 2). Anti-dsDNA positivity rates were calculated as 7.43%, 3.35% and 0.59% for 2011, 2010 and 2009 respectively. Furthermore, anti-dsDNA results were shown statistical significant difference in study period ($p<0.05$). A total of 2 samples were found positive both ANA and anti-dsDNA. There is no statistically significant correlation between ANA and anti-dsDNA positivity (Figure 1,2). Agreement between ANA and anti-dsDNA is generally marginal.

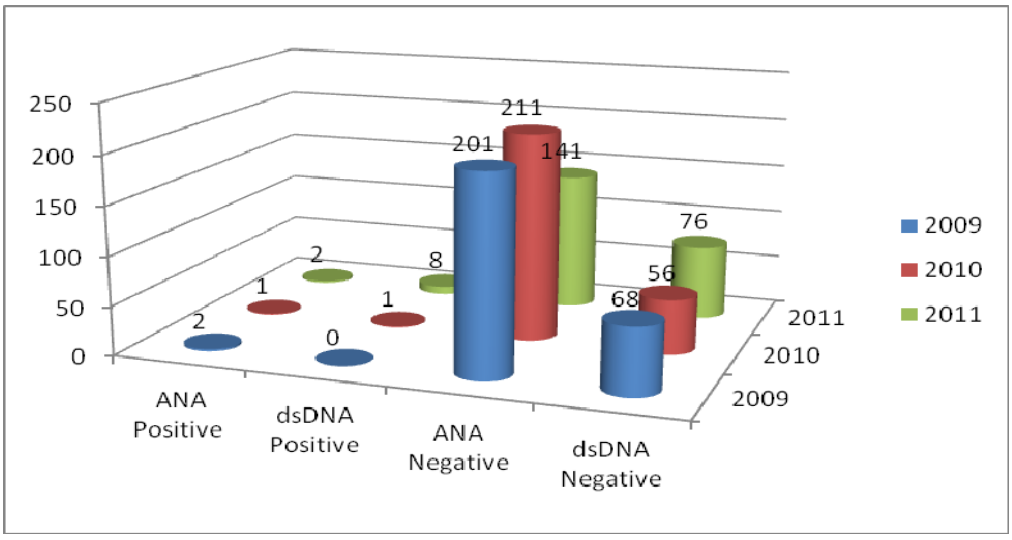

Fig. 1. Male dsDNA- ANA test result Comparison Chart.

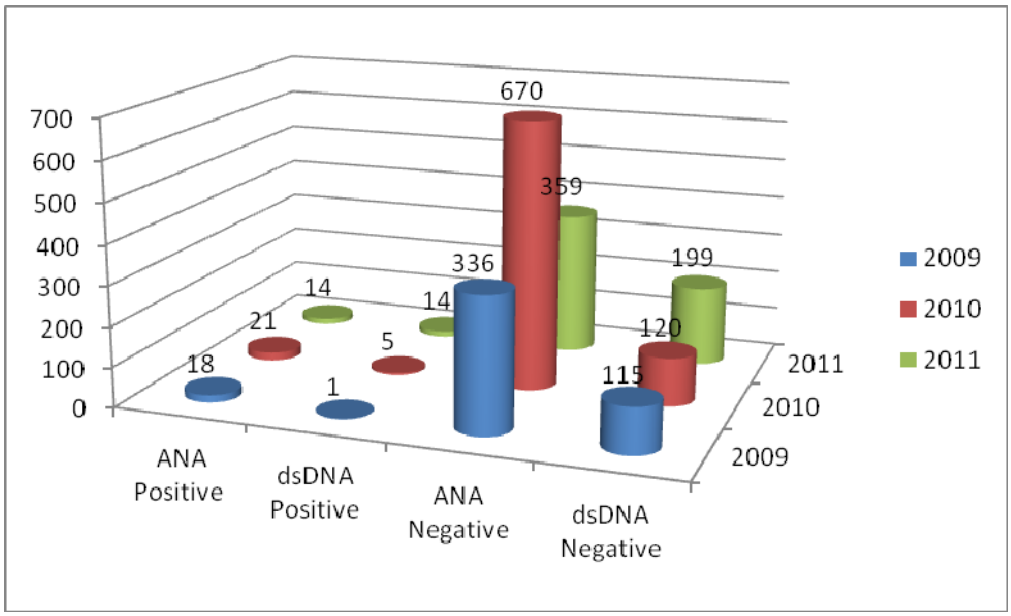

Fig. 2. Female dsDNA - ANA test result Comparison Chart.

4. Discussion

The accurate diagnosis of a patient with suspected autoimmune disease of these disorders depends on the evaluation of 4 parameters, namely clinical findings, histopathology, tissue immunofluorescence, and serologic testing. The presence of ANA and anti-dsDNA is one of the diagnostic criteria for SLE by the American College of Rheumatology (ACR). In routine practice, clinical diagnostic laboratories use the ELISA (9,10). But, there are several parameters that indicate the value of a certain fluorescent technique. These include sensitivity, specificity, positive predictive value, negative predictive value, and benefit (11).

ANA positivity rate in patient with suspected autoimmune disease were found as 8.7-34.4% in published study in our country (12,13). In the present study, results show that the ANA positive rate was fewest (2.96%) in patient with suspected autoimmune disease. This result has had confusion with literature. May be, ANA prevalence was not established by ELISA methods in our region. Because of each method has a different restrictive criterion. Other expectation of this decrease the frequency of ANA positive had been due to inappropriate examination request. In this case, the problem can be solved in two steps: first, ANA screen test should be followed up with fluorescent tests to provide accurate information about autoimmune disease. In a second step, can be used guidelines for appropriate request for diagnostic tests.

In our study; evaluated anti-dsDNA results were shown incompatible with the literature. ANA and anti-dsDNA positivity could be detected only in two patients, and other anti-dsDNA positive patients were not shown ANA positivity simultaneously. Contrary to expectation, there is no correlation between ANA and anti-dsDNA positivity. Additionally, anti-dsDNA results were shown statistical significant difference in study period ($p<0.05$). We consider that this result has a relationship with the inappropriate request of anti-dsDNA.

Finally, in our ANA and anti-dsDNA results has no correlation, but with this study, ANA and anti-dsDNA data were evaluated for the first time in our region. Because of these major disadvantages for ELISA, ANA screen tests have to do with fluorescent technique. The fluorescent ANA test is a very good screening test for most of the previously discussed antibodies (14). But, anti-dsDNA assessment will be conducted with ELISA for its higher sensitivity (10).

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