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ANTI-NUCLEAR ANTIBODY (ANA) PATTERNS IN EGYPTIAN SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract

In fact autoantibodies are immunoglobulins against self-antigens that are known as endogen antigens. Systemic lupus erythematosus (SLE) is an occasion that the body begins a fight against its own cells and organs. There are different types of autoantibodies that have been identified in recent years. The most familiar is anti-nuclear antibodies (ANA). They are created against cell nuclei, and one of the methods used for its detection and its pattern determination is indirect immunofluorescence test (IIF). IIF was used for that purpose in our study, and concluded that ANA positivity rate was higher in female patients than the male ones, where ANA was positive in 36.1% of the male patients and 48.2% of female patients. Also, the most frequent ANA patterns in SLE patients were speckled pattern, where 21.6% & 41.3% of male and female patients, respectively, have speckled pattern of ANA.

Key words: Egypt, Systemic lupus erythematosus, Anti-nuclear antibody

Introduction

Systemic lupus erythematosus (SLE) is a multisystem heterogeneous autoimmune disorder with wide clinical and laboratory manifestations (D'Cruz *et al,* 2007). SLE is characterized by exacerbations and remissions, with development of new organ manifestations or progression of existing manifestations (Petri, 2007). Previous studies have reported the clinical features of SLE based on sex, age at the disease onset, individual autoantibody prevalence and autoantibodies clusters (Cojocaru *et al*, 2011).

Fawzy *et al*. (2016) in Egypt reported that SLE is a multisystem involvement, includeing the gastrointestinal (GI) tract, with a significant variation in the clinical presentation and severity of GI disorders. Such autoantibodies are immunoglobulins, but are targeted against self-antigens known as endogenous protein antigens. The self-antigens might be enzymes, cell receptor, phospholipids and nucleic acids (Muro *et al,* 2005). Anti-nuclear antibodies (ANA) are created against own cell nucleus proteins. Several studies tried to explain how the body-fight itself; it was reported that this may be due to infection, environmental factors or genetical link. These autoantibodies interfere in many SLE manifestations (Heffernan *et al,* 2001; Fritzler *et al,* 2003; Muro *et al,* 2005). The detection of autoantibodies in serum samples of patients plays an important role in diagnosis and in follow-up of the disease in recent years. IIF has been used as a screening test for these autoantibodies, especially for determination of anti-nuclear antibodies (Yumuk *et al,* 2005).

The present study aimed to determine the ANA rate positivity and its patterns in the Egyptian SLE patients using indirect immuno-fluorescence test (IIF), regarding the sociodemographic characters of SLE patients.

Materials and methods

Patients and ANA tests: ANA test results of 300 patients with SLE were selected from the out patients clinics of El-Monira Public Health Hospital and evaluated retrospectively. ANA test (HEp 20-10, EUROIMMUN, Germany) was used in dilution of 1:100 in IIF test (Mengeloglu *et al,* 2014). The slides were prepared according to the instructions of the manufacturer and evaluated under the fluorescence microscope using 40X objective. Intensity of fluorescence was interpreted semi quantitatively based on negative co-

ntrol (0) and positive control (+4).

This study was approved by the MOH Ethics Committee and a signed informed consent was obtained from all the participants.

The included patients underwent regular follow-up for the medical history, physical examination, clinical manifestations and laboratory examinations. Patients who fulfilled at least four of The American College of Rheumatology (ACR) criteria for the SLE classification (Hochberg, 1997) were included.

Statistical analysis: Data were analyzed using IBM SPSS Statistics Version 15.0 (SPSS Inc., Chicago, IL, United States). Descriptive variables were given as numbers and percentages.

Results

Amongst 300 tests, 290 (96.6%) resulted as ANA positive. The mean age was 40.8±5.6 in ANA-positive patients and was 39.3±11.6 in ANA-negative individuals. Amongst the patients, 97 (32.3%) were males and 203 (67.6%) were females. ANA was positive in 35 (36.1%) of the males and 98 (48.2%) of females; accordingly ANA positivity rate was higher in female patients than the male ones. The most frequent ANA patterns were speckled pattern (21.6% & 41.3% for male and female patients respectively). The ANA levels in SLE patients (2.92±1.0) were significantly higher than that (0.53±0.23) in healthy control group. In SLE patients, the highest value for ANA secretion was 5.51 IU/ml and the lowest value was 1.95 IU/ml with a range 3.56. Details were given in tables (1, 2 3 & 4)

Table 1: Socio-demographic characteristics of SLE patients.

Item	Number	%
Age in years:		
20-30	147	49%
31-45	153	51%
Sex:		
Male	97	32.3%
Female	203	67.6%
Marital status:		
Single	279	93%
Married	21	7%
Educational level:		
Illiterate	14	4.6%
Write and read	39	13%
Moderate	190	63.3%
High	57	19%

Table 2: Median and range of ANA concentration (pg/ml) in SLE patients and healthy controls.

	Group	Median	Mode	Lowest value	Highest value	Range
ANA	SLE patients	2.64	0	1.95	5.51	3.56
	Control	0.54	0.21	0.1	0.87	0.77

Table 3: *P* value for concentration of ANA in SLE patients and healthy controls.

Parameters	Control (M±SD)	SLE patients(M±SD)	*P* value
ANA	0.53±0.23	2.92±1.0*	0.04

*Significant difference (P<0.05).

Table 4: ANA pattern in male and female Egyptian patients.

ANA pattern	Male patients	Female patients
Speckle	21 (21.6%)	84 (41.3%)
Nucleolar	4 (5%)	5 (2.4%)
Homogenous	8 (8.2%)	7 (3.4%)
Perinuclear	1(1%)	2 (0.98%)

Discussion

The SLE is a chronic autoimmune inflammatory disease that can harm any organ of the body. The production of ANA was the prominent character of the disease. Diagnosis is based on differential clinical and laboratory markers, after eliminating other cross skin diseases. SLE classification criteria are used as a guide to help in identification of salient clinical characters when making the diagnosis. Serological findings are criteria in suggesting SLE (Riemakasten and Hiepe, 2013).

ANA test is used as a serologic marker for several autoimmune diseases. ANA are immunoglobulins that bind to antigens expressed within the human cells' nucleus. The most common used test for measuring ANA are indirect immunofluorescence antinuclear antibody test (IF-ANA) and ELISA (Marin *et al*, 2009).

A significant test in diagnosis of autoimmune diseases was indirect immunofluorescence test (IIF). This test can be used in determination of ANA pattern leading to excellent differential diagnosis (Yumuk *et al*, 2005). ANA test has advantage as easiness and low cost, however it need experienced staff to be performed. Also, ANA test has low sensitivity and specificity. Clinicians may be in the need IIF results to support ANA results (Yilmaz *et al*, 2001).

The prevalence of positive ANA in SLE was 90-100% and this autoantibodies prevalence differed between different races (Kasitanon *et al*, 2004). Wichainun *et al*. (2013) reported that ANA and anti-dsDNA gave high sensitivity and high specificity in SLE patients, even when using sera of patients with multiple medical problems as controls. According to the present results, ANA levels in SLE patients (2.92±1.0) were significantly higher than that (0.53±0.23) in the healthy control group. Also, in SLE patients, the highest value for the ANA secretion was 5.51 IU/ml and the lowest value was 1.95 IU/ml with a range 3.56.

SLE is known to be less prevalent in males than in females, but the reason for this sexual predilection is still controversies. However, much argument surrounds the differences in complication of the disease in both sexes. Nevertheless, Yacoub Wasef (2004) reported the elevated SLE frequency among females may be due to differences in the sex hormones' metabolism and/or the GnRH. Although, SLE is less common in males, it tends to be more severe. The author recommended the regular follow-up of the SLE male patients. These findings agreed with the present results, where ANA test was positive in 36.1% of males patients and 48.2% of females; accordingly the ANA positivity rate was higher in female patients than the male ones.

In the present study, ANA positivity rate and ANA patterns in male and female Egyptian SLE patients were evaluated. The most frequently reported patterns were speckled, nucleoli and homogenous patterns. The speckled pattern was 21.6% and 41.3% for male and female patients respectively. 5% of male patients and 2.4% of female patients have nucleolus pattern. Also, 8.2% of male patients and 3.4% of female patients have homogenous pattern. The lowest percent was observed with perinuclear pattern, 1% and 0.98% for male and female patients respectively. The present results agreed with Peene *et al.* (2001) who found that the commonest one was speckled patterns. Yilmaz *et al.* (2004) reported homogenous pattern in more than half of their patients. Mengeloglu *et al.* (2014) found a significant association between ANA positivity and SLE, as the most frequent ANA patterns were coarse speckled pattern in patients were (154 or 31.2%), nucleolar pattern (89 or 18.0%), fine speckled pattern (57 or 11.5%), and speckled (granular) pattern (48 or 9.7%).

No doubt, ANA test was positive in connective tissue disorders, however, it was added that ANA positivity was not absolutely diagnostic. The ANA positivity could be observed in 95% in SLE and mixed connective tissue disorders, and that this rate

could be between 25-70% cases with the Sjögren syndrome, systemic sclerosis and RA (Fritzler *et al*, 2003, Muro, 2005).

Conclusion

The data showed that ANA test was positive in female patients than males, ANA testing proved sensitive in screening SLE, speckled pattern was 21.6% & 41.3% for male and female patients respectively and 5% of males and 2.4% of females showed nucleolus pattern. Also, 8.2% of males and 3.4% of females had homogenous pattern. The lowest percent was with per-nuclear pattern, 1% and 0.98% for males and females respectively.

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