



CORRELATION BETWEEN AUTOIMMUNE DISEASES AND EXTRACTABLE NUCLEAR ANTIGEN ANTIBODIES PANEL AMONG PATIENTS AT KING HUSSEIN MEDICAL CENTER

Lamees Abasi (MD)*, Maha Al-Amr (MD), Nabeeha Abbasi (MD), Osama Rabadi (MD), Shadi Aldaoud (MD), Sahel Haddadin (MD), Mu'taz Halasah (MD), Ahmad Mahasna (MD), Ra'ad Al-omari (MD), Huda Khraisat (MD)

KHMC Amman, Jordan.

*Corresponding Author: Lamees Abasi (MD)

KHMC Amman, Jordan.

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ABSTRACT

Objectives: The production of autoantibodies is one of the characteristics of autoimmune diseases (ADs), autoantibodies against extractable nuclear antigens (ENA) are important category of them that could assess the differentiation between various types of autoimmune disorders. The aim of this retrospective study is to confirm the correlation between ENA panel of patients with their clinical diagnoses. **Patients and Methods:** This study goes over patient's records in the adult medical clinics at King Hussien Medical Center (KHMC), during the time period between July 2020 and July 2021. The study group included 200 patients, aged between 18 and 80, females were 144 and males were 56. Serum samples were collected from these patients, then were investigated for the presence of anti-ENA antibodies using Immunoblot method. **Results:** Of the 200 samples, (47%) were positive for anti-ENA. The highest percentage in the study group was anti-SSA/Ro autoantibody which revealed (44%). (29%) of the patients who were negative for anti-ENA, were diagnosed to have an AD, on the other hand, (78%) of the patients who were anti-ENA-positive were diagnosed to have an AD. Systemic lupus erythematosus (SLE) was the highest prevalence in the anti-ENA positive patients. **Conclusion:** The correlation between anti-ENA antibodies and the diagnosis of autoimmune disease was significant. As a consequence, the anti-ENA antibodies test is a worthy indicator to support in the clinical diagnosis of patients with autoimmune diseases.

KEYWORDS: Autoantibodies, autoimmune diseases, extractable nuclear antigens, panel.

INTRODUCTION

Autoimmune diseases are recognized by attacking of the immune system against self-antigens by autoantibodies production, which display clinical importance when related with other disease symptoms.^[1,2]

Several factors can cause autoimmune diseases and include an extensive variety of environmental and genetic factors.^[3]

In the late 1940s, the initial autoantibodies were detected, when rheumatoid factors (RFs) and antinuclear antibodies (ANAs) were defined as serum factors that could bind immunoglobulins and nuclear antigens.^[4,5] They are considered as diagnostic tests of Rheumatoid arthritis (RA) or SLE respectively, as they contribute to disease pathogenesis. Autoantibodies have an essential role in the pathogenesis of different diseases as they cause tissue injury and systemic inflammation.^[6]

Autoimmune disease diagnosis starts with detection of ANA, followed by further investigation of extractable nuclear antigens (ENA), which is described as the most common antigens in autoimmune diseases.^[7,8]

Anti-ENA panel detect a group of specific autoantibodies, containing anti-dsDNA anti-SSA/Ro, anti-SSB/La, anti-Sm, anti-Scl-70, anti-Jo-1, anti-RNP anti-CENP-B, and anti-NUC. ENA autoantibodies are identified using many methods, including enzyme-linked immunosorbent assay (ELISA), immunoblot, hemagglutination, immunodiffusion and counterimmunoelectrophoresis. These techniques may vary in the results because they have different sensitivity and specificity.^[9-11]

ENA autoantibodies may discriminate diverse varieties of autoimmune rheumatic diseases (ARD). For example, Sjögren's syndrome (SS) is diagnosed by the presence of antibodies directed against SS-A (Ro) or SS-B (La) ribonucleoproteins and positive anti histidyl-sRNA

synthetase (Jo-1) antibodies is a specific immunological characteristic of polydermatomyositis. The detection of anti-centromere antibodies (CENP-B) or topoisomerase 1 (Scl-70) supports the diagnosis of systemic sclerosis.^[12-14]

The presence of anti-dsDNA or anti-Sm are diagnostic criteria of SLE. Also, the presence of anti-ribonucleoprotein (RNP) antibodies is a diagnostic test of mixed connective tissue disease (MCTD).^[15,16]

The aim of this study is to evaluate the correlation between anti-ENA antibodies and the diagnosis of autoimmune disease, and to detect their clinical significance.

MATERIALS AND METHODS

This study goes over patient's records in the adult medicine and rheumatology clinics at King Hussien Medical Center (KHMC), suffering from signs and symptoms related to autoimmune rheumatologic diseases, during the time period between July 2020 and July 2021. The study group included 200 patients, aged between 18 and 80, females were 144 and males were 56. Serum samples were collected from these patients, then were investigated for the presence of anti-ENA antibodies using Immunoblot method by EUROLINE ANA Profile 3 (IgG) test kit, following the manufacturer instructions. EUROIMMUN Medizinische Labordiagnostika AG, 23560 Lubeck (Germany). Seekamp 31.^[17]

RESULTS

Of the 200 samples, 94 patients (47%) were positive for anti-ENA. The anti-ENA-positive patients aged from 18 to 80 years, averaging 37.7 years, with a female prevalence of (84.2%).

The highest percentage in the study group was anti-SSA/Ro autoantibody which was positive in 41 patients

(44%). The other most commonly detected autoantibodies were SS-B /La (n=10, 10.6%), anti-Scl-70 (n=9, 9.6%), anti-nRNP/Sm (n= 8, 8.5%), anti- histone (n=7, 7.4%), anti-Nucleosome (n= 5, 5.4%), anti-Jo-1 (n=4, 4.3%), anti-Rib. P-protein (n=3, 3%), anti- dsDNA (n=2, 2.1%), anti- CENP-B (n= 2, 2.1%), anti-PCNA (n= 1, 1%), anti-PM-Scl (n=1, 1%), anti- AMA-M2 (n= 1, 1%). The distribution of anti-ENA autoantibodies in anti-ENA positive patients is shown in Table (1).

Twenty nine percent of the patients who were negative for anti-ENA, were diagnosed to have an AD. On the other hand (78%) of the patients who were anti-ENA-positive were diagnosed to have an AD.

SLE was the highest prevalence (85%) in the anti-ENA positive patients, followed by Sjögren's syndrome (5%), Scleroderma (4%), Rheumatoid arthritis (2%), Polymyositis (2%), MCTD (1%) and Behçet's disease (1%). The distribution of ADs and autoantibodies against ENA are shown in Table (2).

Table (1): The distribution of anti-ENA autoantibodies in anti-ENA positive patients.

Anti-ENA autoantibodies	Number & Percentage
anti-SSA/Ro	(n=41, 44%)
SS-B /La	(n=10, 10.6%)
anti-Scl-70	(n=9, 9.6%)
anti-nRNP/Sm	(n= 8, 8.5%)
anti- histone	(n=7, 7.4%)
anti-Nucleosome	(n= 5, 5.4%)
anti-Jo-1	(n=4, 4.3%)
anti-Rib. P-protein	(n=3, 3%)
anti- dsDNA	(n=2, 2.1%)
anti- CENP-B	(n=2, 2.1%)
anti-PCNA	(n= 1, 1%)
anti-PM-Scl	(n= 1, 1%)
anti- AMA-M2	(n= 1, 1%)

Table 2: Distribution of ADs and autoantibodies against ENA.

AD in Anti-ENA-positive patients	Extractable Nuclear Antigens found
SLE (85%)	SS-A (Ro), SS-B (La), Nucleosome, dsDNA, RNP/Sm, PCNA, Histone, Rib. P-protein
Sjögren's syndrome (7%)	SS-A (Ro), SS-B (La)
Scleroderma (5%)	SS-A (Ro), Scl-70, CENP-B, PM-Scl, AMA-M2
Polymyositis (2%)	Scl-70, RNP/Sm, Jo-1
MCTD (1%)	RNP/Sm

SLE = systemic lupus erythematosus.

MCTD = mixed connective tissue disease.

DISCUSSION

The aim of this study was to evaluate the distribution of anti-ENA autoantibodies in different AD.

Our study showed that 94 patients (47%) were positive for anti-ENA, while a study done by Banhuk FW et al, showed a different result with (20.9%) of their patients

were positive for anti-ENA antibodies. Also, we reported a female predominance of ENA positive patients (84.2%) and that was similar to their results.^[18]

We found that anti-SSA/Ro autoantibody had the highest percentage in the study group with (44%). Similar

results were reported by Lora et al. and Sanchez-Guerrero et al.^[19,20]

SLE was the most encountered AD in anti-ENA positive patients with (85%). A study done by Banhuk FW et al, showed a different prevalence of SLE (33.3%) in anti-ENA-positive patients (18). A higher frequency of SLE patients (50.8%) was reported by Lora et al.^[19]

Our study reported that (29%) of the patients who were negative for anti-ENA, were diagnosed to have an AD. While a study done in Brazil found that (35%) of anti-ENA-negative patients exhibited manifestations of AD.^[18] These findings explain the significance of the ANA test, which is the screening test for autoimmunity.^[11]

The literature reported an association between autoantibodies against extractable nuclear antigens and AD, such as Sjögren's Syndrome and SS-A/Ro or SS-B/La and Scleroderma and Scl-70.^[21,22] Similar findings was found in this study.

Regarding SLE, it was highly associated with autoantibodies against the following antigens: SS-A (Ro), SS-B (La), Nucleosome, dsDNA, RNP/Sm. A study done by Aganovic-Musinovic I et al, who found that anti-Sm/RNP is a very significant antibody for SLE diagnosis followed by Jo-1.^[23]

CONCLUSION

The correlation between anti-ENA antibodies and the diagnosis of rheumatological autoimmune disease was significant. As a consequence, the anti-ENA antibodies test is a worthy indicator to support in the clinical diagnosis of patients with autoimmune diseases.

It is crucial to describe the existence of autoantibodies that are specific to autoimmune diseases by distinctive methods in ANA-positive patients.

Experts that work with ANA and anti-ENA investigations must implement a precise analysis of the models that assist in the clarification of the results to successfully aid in the clinical diagnosis and follow-up of the patients.

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