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EVALUATION OF AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS AND THEIR CLINICAL CORRELATION

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ABSTRACT
Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease with great variety of clinical presentations and demonstration of auto-antibodies. Very few studies have been conducted to observe the clinical correlation of individual auto-antibody. The present study is done to observe the frequency of multiple auto-antibodies, and analyze their correlation with clinical presentations.

KEYWORDS: Systemic lupus erythematosus, anti-nuclear antibody, anti-double stranded DNA antibody, anti-cardiolipin antibody.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease that involves almost all the organs in the human body. Ninety percent of patients are woman of child-bearing years. People of both sexes, all ages and all ethnic groups are susceptible.[1] In SLE, the organs undergo damage mediated by tissue-binding autoantibodies and immune complexes.

The great diversity of clinical manifestations in SLE, ranging from mild arthritis through pericarditis, and nephritis to life-threatening neuropsychiatric manifestations, is accompanied by a huge number of autoantibodies(1). While many autoantibodies are detected in patients with rheumatoid arthritis or polymyositis, there is no other autoimmune disease similar to SLE with regard to the number of autoantibodies found.

B-lymphocytes from patients with SLE display a lack of self-tolerance, and an inappropriate overproduction of antibodies.[2] The presence of anti-nuclear autoantibodies (ANA) is the immunological hallmark of SLE. In clinical practice, ANA testing is often used as a part of initial investigative screen. A positive ANA is a sensitive test, found in more than 95% SLE patients[3], but the presence of anti-double stranded (anti-ds) DNA antibodies is a much more specific finding. Anti-ds DNA antibodies are seen in approximately 70% of patients with SLE.[4]

The precise role that anti-ds DNA antibodies play in lupus remains an area of great interest. Serial serum concentrations of these antibodies reflect disease activity in many patients, but not all. Instead of simply acting as a disease marker, it is now clear that some anti-DNA antibodies are, in some way, directly pathogenic.[5]

In addition to anti-DNA antibodies, a variety of other autoantibodies are often detected, e.g. anti-Ro/SSA, anti-La/SSA, anti U1 sn-RNP/anti-RNP. The antigens targeted may be associated with patient ethnicity (for example, increased levels of anti-Smith antibodies seen in Afro-Caribbean patients)[6], or particular disease manifestations (for example, anti-Ro antibodies seen in association with photosensitive rashes).[7] Finally, patients with lupus are often found to have positive anti-cardiolipin antibodies (ACLA), with or without the related clinical syndrome.[8,9,10]

Aims and objectives

- To study the prevalence of different autoantibodies in patients with SLE
- To analyze the correlation of the autoantibodies with the clinical manifestations.

MATERIALS AND METHODS

Sera were collected from patients with clinical manifestations of SLE, and diagnosis was made using the American College of Rheumatology revised criteria (1997) for the classification of SLE.

Table 1: 1997 Update of the 1982 American College of Rheumatology revised criteria for classification of systemic lupus erythematosus[12]

1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3.Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	• Pleuritis--convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion, or, • Pericarditis--documented by electrocardigram or rub or evidence of pericardial effusion
7. Renal disorder	Persistent proteinuria > 0.5 grams per day or > than 3+ if quantitation not performed, or, ceellular casts(may be red cell, hemoglobin, granular, tubular, or mixed)
8. Neurologic disorder	Seizures or psychosis in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance,
9. Hematologic disorder	Hemolytic anemia with reticulocytosis, or, leucopenia < 4,000/mm^3 on ≥ 2 occasions, or, Lyphopenia < 1,500/ mm^3 on ≥ 2 occasions, or, thrombocytopenia <100,000/ mm^3 in the absence of offending drugs
10. Immunologic disorder	• Anti-DNA: antibody to native DNA in abnormal titer, or, • Anti-Sm: presence of antibody to Sm nuclear antigen, or, • Positive finding of antiphospholipid antibodies on: • an abnormal serum level of IgG or IgM anticardiolipin antibodies, • a positive test result for lupus anticoagulant using a standard method, or • a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Positive antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

The classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, aperson is defined as having SLE if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation. The samples for control population were collected from healthy volunteers, after excluding the presence of any dermatological and auto-immune disorder. The different autoantibodies were detected using enzyme-linked immunosorbent assay (ELISA) methods.

OBSERVATIONS

A total number of 108 cases were collected, among which 24 were male and 84 were female, with a male:female ratio being 1:3.5. The cases were aged between 11 to 70 years, with a mean age of distribution of 29.6 years. The mean age of the male cases was 38.7 years, which was much higher than the female cases (mean age 27.1 years). A total number of 64 controls were collected, among which 21 were male and 43 were female (male:female =1:2.04).

TABLE 2: Frequency of different auto-antibodies among the cases.

Name of the antibody		Number of positive cases	Percentage of positive cases
Anti-nuclear antibody (ANCA)		108	100
Anti-ds DNA		79	73.1
Anti-cardiolipin (ACLA) antibody		60	55.5
Anti-neutrophilic cytoplasmic antibody (ANCA)	cytoplasmic ANCA (cANCA) or anti-proteinase 3 (anti-PR 3)	9	8.3
	perinuclear ANCA (pANCA) or anti-myeloperoxidase (MPO)	3	2.8
Anti-Smith antibody (anti-Sm)		24	22.2
Anti-U1 snRNP		57	52.7

All 108 cases, in the study, showed positivity for ANA, while 73.1% were positive for anti-ds DNA. 55.5% cases had a positive reaction for anti-cardiolipin (ACLA) antibody. Only 11.1% cases were positive for ANCAs. Among the ANCAs, anti-PR3 antibody was most frequent, accounting for 8.3%. We also observed a 22.2% positivity for anti-Sm and 52.7% positivity for

anti U1 snRNP.None of the antibodies was detected in control population.

TABLE 3: Frequency of different clinical presentations among the cases and their correlation with different auto-antibodies

Clinical presentation	Number of Cases with the clinical presentation	Antibody positivity among the cases with the clinical presentation			
		Anti-ds DNA	ACLA	SSA	Anti-RNP
Arthritis	78 (72.2%)	39 (50%)	39 (50%)	39 (50%)	36 (46.2%)
Malar rashes	48 (44.4%)	15 (31.2%)	33 (68.8%)	18 (37.5%)	18 (37.5%)
Photosensitivity	30 (27.8%)	6 (20%)	21 (70%)	12 (40%)	15 (50%)
Oral ulcers	21 (19.4%)	9 (42.8%)	6 (28.6%)	12 (57.1%)	15 (71.4%)
Renal diseases	18 (16.7%)	6 (33.3%)	9 (50%)	6 (33.3%)	12 (66.7%)

Arthritis (72.2%) was the commonest presentation, followed by malar rashes (44.4%), photosensitivity (27.8%), oral ulcers (19.4%) and renal diseases (16.7%). Arthritis was most commonly associated with anti-ds DNA, SSA and ACLA (50% of the cases each), followed by anti-RNP (46.2%). Malar rashes were reactive for ACLA (68.8%), anti-RNP (37.5%), SSA (37.5%) and anti-ds DNA (31.2%). Photosensitivity showed positivity for ACLA (70%), anti-RNP (50%) and SSA (40%), and oral ulcers were positive for anti-RNP (71.4%), SSA (57.1%) and anti-ds DNA (42.8%). Discoid rash was frequently associated with anti-RNP (85.7%), anti-ds DNA (71.4%) and SSA (57.1%). Association of renal diseases and anti-RNP was found in 66.7% cases, followed by ACLA (50%), anti anti-ds DNA and SSA (33.3% each).

DISCUSSION

SLE is an autoimmune disease affecting almost every human organ-system. Women of child-bearing age are mostly affected, although people of both sexes and any age can be affected(1). In our study, male-female ratio was 1:3.5. The youngest patient was 11 years old, while the oldest being 70 years old. The female patients were much younger (Mean age 27.1 years) in comparison to the male patients (Mean age 38.7 years). SLE is unique in having a wide variety of clinical manifestations, as well as, demonstration of a great number of autoantibodies.[1]

ANA testing involves the use of indirect immunofluorescence to detect antibodies that bind to various nuclear antigens. In SLE and drug-induced lupus the sensitivity of ANA testing approaches 100 percent, with a specificity of approximately 90%.[3,4,14,15] ANA testing is a part of an initial investigative screen when SLE is suspected. All the cases, in our study, were positive for ANA.

Anti-ds DNA antibodies are highly specific for SLE. However, only 60-70% of SLE patients turn out to be positive for anti-ds DNA.[3,5] Testing for anti-ds DNA may be useful in patients with a positive ANA test and clinical suspicion for SLE. The presence of anti-ds DNA tends to correlate with lupus nephritis, and the anti-ds DNA level often correlates with disease activity in SLE. In our study, 73.1% cases were reactive for anti-ds DNA. A higher occurrence of anti-ds DNA positivity was observered in patients with arthritis and lupus nephritis.

Anticardiolipin antibodies (ACLA) can be detected in about 40% SLE patients, ranging from 23-82%. Although ACLA is proved to be associated with vascular thromboses, including cerebral infarction and spontaneous abortion, its role in lupus nephritis is controversial.[8,9,10,11] 55.5% of our cases were positive for ACLA. All the cases with vasculitis and recurrent spontaneous abortions, and half of the lupus nephritis cases were reactive for ACLA.

ANCAs are directed against a number of antigens located in the cytoplasm of neutrophils. ANCA has two variants: cytoplasmic ANCA (cANCA) against enzyme proteinase 3, and perinuclear ANCA (pANCA) against enzyme myeloperoxidase (MPO). cANCA is highly specific and sensitive for detection of Wegener's granulomatosis, while pANCA is frequently associated with microscopic polyangiitis and necrotizing glomerulonephritis.[13,14] However, the sensitivity of pANCA for these diseases is very low. Only 8.3% and 2.8% cases of our study population were positive for cANCA and pANCA, respectively. This finding is contradictory to a previous study which documented a much higher (37.3%) ANCA positivity among SLE cases. That study also concluded that ANCA in SLE may be used along with clinical and histopathological assessment to differentiate vasculitides in lupus nephritis cases from lupus without nephritis. However, we failed to demonstrate any correlation between ANCA and clinical manifestations in our study.

Several autoantibodies against small nuclear ribonucleoproteins (anti-sn RNPs) have been described. Anti-smith (anti-Sm) antibodies are specific for SLE, although they are detected in only 20-30% cases.[15,16] Anti U1 snRNP is present in 30-40% of SLE cases, and

is associated with disease activity, myositis, esophageal hypomobility, sclerodactyly, Raynaud's phenomenon, arthralgias and arthritis. We observed a 22.2% positivity for anti-Sm and 52.7% positivity for anti U1 snRNP among our study cases, and anti U1 snRNP were associated with nephritis and recurrent fetal loss.

Anti-Ro (anti SS-A) and anti-La (anti SS-B) are commonly identified in patients with Sjögren's syndrome, and their presence is associated with extraglandular manifestations of the disease. Anti-Ro activity is also found in approximately 40% of SLE patients, and is associated with photosensitive skin rash, pulmonary disease and lymphopenia.[7,17,18] Anti-La activity is detected in 10-15% of patients with SLE, and is associated with late-onset disease, secondary Sjögren's syndrome and neonatal lupus syndrome.[15] Anti-Ro and anti-La were positive in 41.6% and 13.8% of our study cases, respectively, and a higher incidence of anti-Ro positivity was obtained among patients with photosensivity (61.5%), discoid rash (57.1%) and malar rash (37.5%).

Anti-Jo 1 (anti-histidyl tRNA synthetase) antibody was found in 30% of patients with polymyositis or dermatomyositis. It is associated with pulmonary fibrosis and Raynaud's phenomenon.[4] Anti-topoisomerase I (anti-Scl 70) is highly specific and is found in 22-40% of patients with scleroderma.[12] A very low incidence of anti-Jo (5.5%) and anti-Scl 70 (8.3%) is observed among our study cases, however, all the cases with vasculitis were reactive for anti-Scl 70.

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