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ORIGINAL ARTICLE

Clusters of Antinuclear Antibodies in Patients with Systemic Lupus Erythematosus from Tamil Nadu

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ABSTRACT
The frequencies of antinuclear antibodies (ANA) have been associated with Systemic Lupus Erythematosus (SLE). In this study, we had evaluated the frequency of antinuclear antibodies associated with SLE by Line Immuno Assay (LIA). Clusters of antinuclear antibodies were identified using hierarchical clustering method. A total of 100 SLE patients (96 females, 4 males) with age ranging between 20 and 67 years were enrolled in this study. Five clusters of antibodies were identified in 100 SLE sera by cluster analysis. Cluster 1 contains antibodies to dsDNA, nucleosomes and histones; cluster 2 contains antibodies to CENP B, PCNA and Scl-70; cluster 3 contains antibodies to Jo-1, PM-Scl, SS-B, AMA-M2, Sm, Ribosomal P protein and SS-A; cluster 4 and 5 contain antibodies to Ro-52 and nRNP respectively. These results suggest that clusters of antinuclear antibodies were associated with SLE disease progression.
Keywords:, *Antigens, Antinuclear antibody, Erythrocyte sedimentation rate , Systemic Lupus Erythematosus (SLE)*

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by the presence of many different auto-antibodies against self antigens [1, 2]. The antigen-antibody complexes present in blood vessels and filter organs cause inflammation [3-7]. Following inflammation, a wide spectrum of clinical manifestations is observed in any of the functioning systems such as cardiovascular, renal and brain [8]. Different clusters of antibodies have been found in patients with SLE [9] and henceforth act as an important diagnostic marker [10-15]. Moreover, specific antinuclear antibodies are associated with different clinical manifestations of SLE [16-19].

There are several study has been reported the prevalence of ANAs in SLE patients from different part of world. Arbuckle et al. has reported that ANAs were present in 78% of the SLE cases from northern Sweden, among those 55% were anti-dsDNA antibodies and 47% were anti- Ro/SSA antibodies. Eriksson et al. demonstrated the autoantibodies against nuclear antigens and a mean (±SD) of 5.6 ± 4.7 years before the onset of symptoms and 8.7 ± 5.6 years before diagnosis in 63% of the individuals who subsequently developed SLE.

In the present study, we investigated the clusters of antinuclear antibodies among patients with SLE. As an extended study, we have also demonstrated the association between antinuclear antibodies and different clinical measures in those patients.

MATERIAL AND METHODS

Study population

One hundred patients with SLE were included in this study. Blood (2-3ml) was collected from SLE patients who attended outpatient wards of Rheumatology Department, Government General Hospital, Chennai, India. The samples were collected from those patients who fulfilled at least 4 criteria of the American College of Rheumatology (ACR) [20]. Serum was separated and stored immediately in aliquots at -80˚C until use. This study was approved by the Ethical Committee of Madras Medical College & Hospital, Chennai, Tamil Nadu and an informed written consent was obtained from each participant.

ANA profiles by LIA

Antibodies against nuclear antigens were analyzed by the Line Immuno Assay (Meheus, 1999, Damoiseaux et al.) using the kit supplied by EUROIMMUN ANA PROFILE EUROLINE, UK. This assay includes 14 nuclear antigens viz. nRNP, Sm, SS-A(SS-A native & Ro-52), SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-protein and AMA-M2. This assay was performed with the patient's sera according to the manufacturer's instructions. In brief, to each precoated nuclear antigen channel 1.5 ml of sample buffer was added and incubated at room temperature for 5 minutes. Following which, the buffer was aspirated out and 1.5ml of diluted serum sample was added and incubated for 30 minutes at room temperature. After washing with 1.5ml of wash buffer, 1.5ml of diluted enzyme conjugate, substrate solution was also added and incubated for 30minutes. Using stop solution (distilled water) the reaction was stopped and measured by Euroline Scan.

Statistical analysis

Cluster Analysis through average linkage was performed using SPSS Package 22. We followed hierarchical clustering method to group similar variables together. For obtaining the values, significance test and Pearson's correlation were analyzed. The values of each parameter were represented as Mean±SD and their corresponding *P-value ≤0. 05* were considered to be statistically significant.

RESULTS

Demography

A total of 100 SLE patients (96 females and 4 males) with age ranging between 20 and 67 years were analyzed. The mean age of the SLE patients included in this study was 39 years. In age wise distribution analysis, a maximum number of SLE patients (nearly 34%) was observed in 21-30 years followed by 31-40years (30%), 41-50 years (17%), 51-60 years (14%) and 61-70 years (5%). Figure 1 shows clinical manifestations of SLE patients. Clinical manifestations arthritis (54%), photosensitivity (49%), skin rash (46%), and discoid rash (41%) were seen more common in those SLE patients (Figure 1).

Prevalence of ANA

The frequency of specific antigen-antibody interaction obtained by ANA profiling is shown in Figure 2. While RNP/Sm and Ro52, tops the list with 48 and 43 positive cases, respectively CENP B and PCNA showed the least frequency with 3 and 1 positive cases respectively.

Presence of clusters of antibodies

Antibody cluster analysis had shown that there were five clusters of antibodies with 100 SLE sera studied. Cluster 1 contains antibodies to dsDNA, nucleosomes and histones; cluster 2 contains antibodies to CENP B, PCNA and Scl-70; cluster 3 contains antibodies to Jo-1, PM-Scl, SS-B, AMA-M2, Sm, Ribosomal P protein and SS-A; cluster 4 and 5 contain antibodies to Ro-52 and nRNP respectively. The number of SLE cases in each cluster and their corresponding cluster dendrogram were given in Figure 3.

DISCUSSION

In this study SLE positive cases were more prevalent in the age group 21-30, and the median age of disease onset is 21 years. According to Kumar [21] the median age of onset of SLE in Indian population is 24.5 years. Masi *et al.* [22] observed a median age of SLE disease onset between 30 and 31 years in Atlanta, USA. Malaviya *et al*. [23] from New Delhi reported a median age of SLE disease onset is between 24 and 26 years.

In our study, more females (96) were affected with SLE when compared to males (4). The female to male ratio of 24:1 is found among SLE cases in this study. Malaviya *et al*. [23] reported a female to male ratio of 8:1 among SLE cases. Kumar (2002) reported that SLE affects predominantly women in their reproductive years and a sex ratio (F/M) of 11:1 among SLE cases in his study. The present study does differ from earlier findings [21-23] in the median age of onset of SLE and in the female to male ratio of SLE cases. The increased frequency of SLE among females in this study is might be due to hormonal effects.

Moreover, arthritis, photosensitivity, skin rash, discoid rash were more commonly associated in our study patients. Pradhan et al. had reported that arthritis, malar rash and/or discoid rash were more commonly associated with SLE which was similar to our findings. Results of our study shows antibodies to nRNP followed by Ro52-recombinant were seen commonly in SLE patients who were risk for arthritis.

In the present study, antinuclear antibodies were found in 100 SLE patients. High frequencies of antinuclear antibodies to nRNP (*48%*) followed by Ro52-recombinant (*43%*), dsDNA (*37%*), histones (*35%*), nucleosomes (*34%*), SSA-Native (*33%*), Sm (*22%*), ribosomal P protein (*28%*), AMA-M2 (*16%*) and SS-B (*11%*). Eriksson et al. stated that the seroprevalence of ANAs before onset of disease symptoms in individuals who later developed SLE. They found ANA (45.7%), dsDNA (20%), Ro/SSA (20%), Histone (14.3%), RNP (11.4%), La/SSB (8.6%), Jo-1 (8.6%), Scl-70 (5.7%), Sm (2.9%) and CENP B (2.9%).

Hoffman *et al.* [9] also studied associations between antinuclear antibodies and signs/symptoms in European patients with SLE. They observed that the frequencies of ANA were: Ro-52 (31.5%), dsDNA (29.1), Histones (28.5%), Sm (28.1), nRNP (18.3%), CENP B (2.6%), Scl-70 (2.6%) and Jo-1 (1.3%). Results of the present study differ from earlier findings [9] in the antinuclear antibody prevalence.

Interestingly, 8% of our study population shows positive for PM-Scl, whereas 7% were antibodies to Jo-1, 4% were anti-Scl-70, 3% were anti-CENP B and 1% was PCNA antidies. Presence of PM-Scl antibodies was commonly associated with polymyositis, systemic sclerosis and overlap syndromes. Several previous studies also reported that the presence of Pm/Scl antibodies associated with SLE. Hanke et al. demonstrated a Pm-Scl antibody positives in 5.5% of SLE. Warner and Greidinger state 42% SLE cases were positive for dsDNA antibodies and Pm-Scl. In our study we found only 3 of 7 Pm/Scl patients were positive for dsDNA antibodies. Whereas 4/7 Pm-Scl patients were negative for dsDNA antibody. Cluster dendrogram shows Pm-Scl antibodies were clustered with antibodies to CENP B, PCNA, Scl-70 and Jo-1, but not with dsDNA antibodies. Anti-Pm-Scl positives in patients without anti-ds DNA were likely to develop polymyositis-Scleroderma overlapping syndrome.

Furthermore results of our study revealed a 4% SLE patients were positive for anti-Scl70. Only 2 out of 4 anti-Scl70 patients were anti-ds DNA positives. These findings were Gussin et al. found that 25% of anti-Scl-70 positives in 128 consecutive SLE patients. None of the SLE patient could be classified as also having systemic sclerosis. A significant correlation was also found between the levels of antiScl70 and anti dsDNA antibodies.

Moreover, antinuclear antibody is a good screening test for Systemic Lupus Erythematosus because 95% of patients show a high titer of this autoantibody. A positive result for ANA supports the diagnosis of SLE [23].

The variance of our results from previous studies in values of demography, prevalence of ANA might be due to different ethnic population from various geographical regions of the world.

In conclusion, the results of this study suggested that the presence of antinuclear antibodies clusters plays an important role in the pathogenesis of SLE.

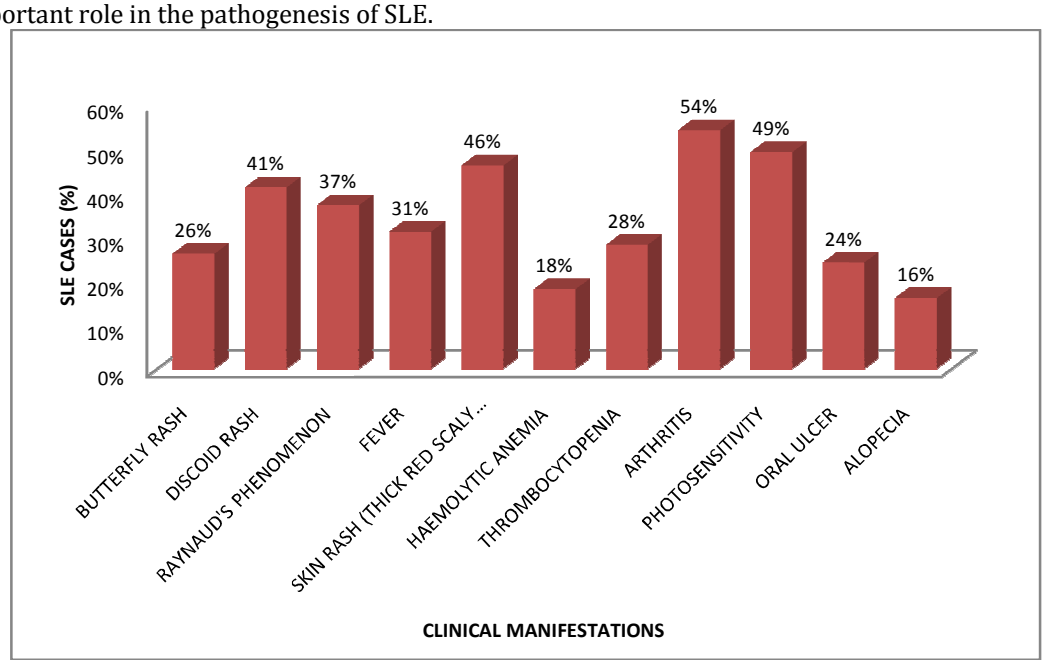

Figure 1. Clinical characteristics of SLE patients

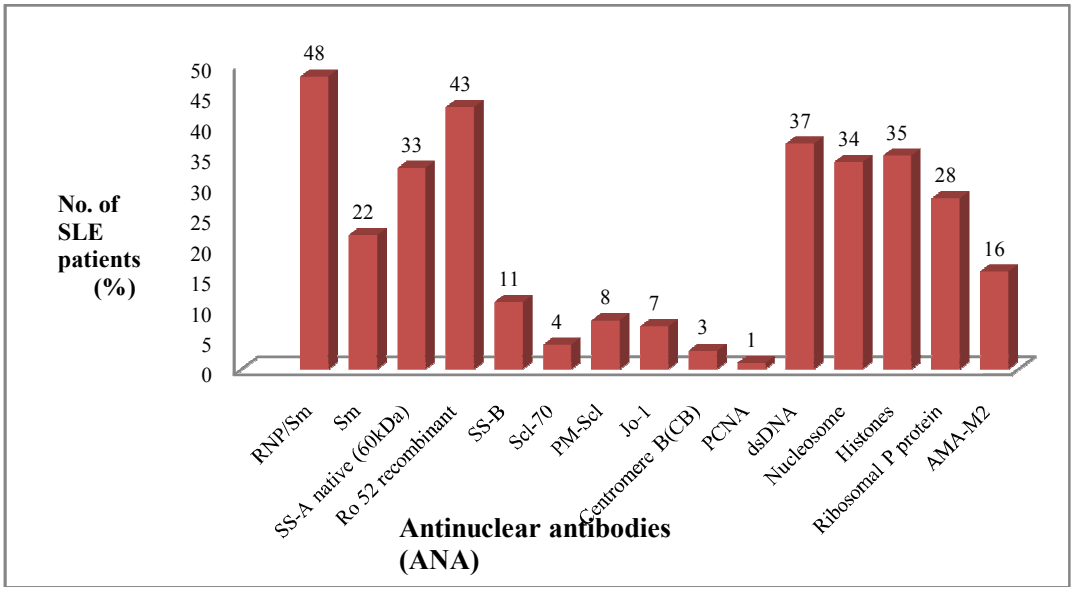

Figure 2. Percentages of antinuclear antibodies differentially expressed in Systemic Lupus Erythematosus patients. The total number of patients tested for presence of antinuclear antibody is 100. RNP: ribonucleoprotein; Sm: Smith antigen; SS-A: soluble substance A; SS-B: soluble substance B; dsDNA: double-stranded DNA; PCNA: proliferating cell nuclear antigen; AMA-M2: anti-mitochondrial M2 antigen.

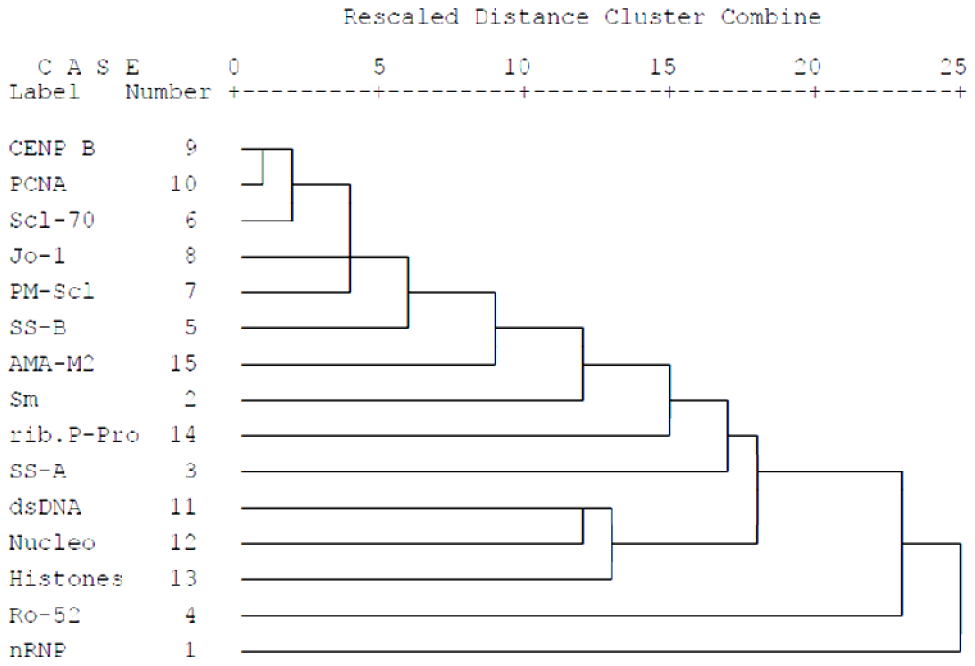

Figure 3. Hierarchical cluster analysis, showing clusters of antinuclear antibodies (dendrogram using average linkage [between groups]). Cluster 1, 2, and 3 are formed initially, whereas cluster 4 consisted antibodies to Ro-52 and cluster 5 consisted antibodies to nRNP do not cluster initially and they are clustered by themselves thereafter. The scale represents the level of distance between the different variables.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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ABBREVIATIONS

ACR, American College of Rheumatology; ANA, antinuclear antibody; CENP B, centromere protein-B; ELISA, Enzyme Linked Immunosorbent Assay; LIA, Line Immune Assay; nRNP, nuclear ribonucleoproteins; PCNA, proliferating cell nuclear antigen; SLE, Systemic Lupus Erythematosus; SPSS, Statistical Package for the Social Sciences.

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