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Antinuclear Antibodies in Thai Patients with Connective Tissue Diseases

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SUMMARY **A study of antinuclear antibodies (ANA) among Thai patients with various connective tissue diseases revealed that the prevalence of ANA was similar to that in other countries, but that the ANA patterns showed interesting contrasts in most diseases. Rather than the predominant homogeneous pattern seen elsewhere in systemic lupus erythematosus and rheumatoid arthritis, the speckled pattern was commonest among Thai patients with these two diseases (67.9% and 76.9% respectively). Patients with scleroderma exhibited a much lower percentage of the nucleolar pattern (17%) than reported elsewhere. The discrepancy between our findings and those from other studies may reflect differences in genetics, the environment or the severity of disease.**

One of the essential laboratory tests that has proved helpful in the diagnosis of connective tissue diseases is the detection of antinuclear antibody (ANA). Patients with this group of disorders characteristically exhibit ANA in their sera.[1] The ANA are a group of antibodies that are reactive against nuclear constituents of mammalian tissue, including deoxyribonucleic acid (DNA), histones, nonhistone acidic proteins and the nucleolus.[1] With the determination of ANA by the indirect immunofluorescent technique, several nuclear staining patterns of ANA can be demonstrated in tissue substrates. Four major patterns frequently observed are the homogeneous pattern, the shaggy or rim pattern, the speckled pattern and the nucleolar pattern (Figure 1). Some of these patterns have certain diagnostic specificities.[2, 3] The first, the homogeneous pattern of nuclear staining, has been correlated with sera containing antibodies to insoluble deoxyribonucleoprotein and histone. These antibodies are mainly found in patients with idiopathic lupus erythematosus (SLE) and drug induced lupus.[3-5] The second, the rim or peripheral pattern has been correlated with antibodies to native DNA. These antibodies are almost exclusively found in sera from patients with SLE, particularly in the active stage.[3-7] The third pattern, the speckled pattern of nuclear staining, is not specific for any disease, since it is produced by a variety of antibodies directed against a number of nonhistone acidic proteins extracted from the nucleus.[5, 8-11] Among these proteins, only a few have been identified and some have been considered to be of significant diagnostic and prognostic value, For example, anti-Sm antibody is believed to be found only in patients with SLE.[8, 12] The Jo-1 antibody has been reported to be a serologic marker for a subset of polymyositis with interstital pulmonary fibrosis, and the anti Scl-70 antibody serves as a specific disease marker for scleroderma.[13,14] Anti n-RNP antibody has been described in some studies to be associated with a better prognosis in SLE.[11, 15] A different technique, however, is required to identify the antibodies to these nonhistone acidic proteins. The fourth and final pattern to be considered here, nucleolar staining, has been reported to indicate antibody directed against nucleolar ribonucleic acid.[16] It is described mostly for sera from patients with PSS. There are some other nuclear staining patterns that can be seen when substrates besides mouse kidney and rat liver are utilized, but their clinical significance is less defined.

The ANA determination has been established by many laboratories to be a sensitive and specific screening

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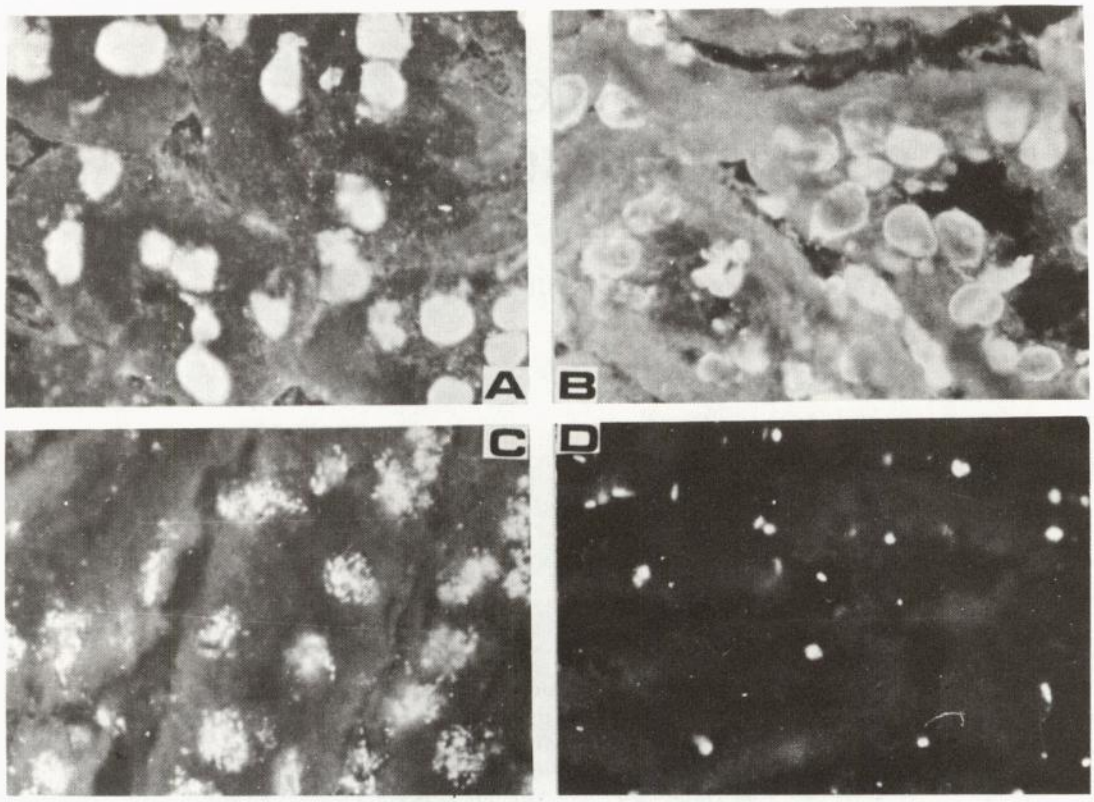

Fig. 1 Antinuclear antibodies utilizing mouse kidney as substrate. A. Homogeneous pattern, B. Rim or shaggy pattern, C. Speckled pattern and D. Nucleolar pattern.

test for patients suspected of having connective tissue diseases.[17] The prevalence of ANA and the frequency of different patterns among various connective tissue diseases has been described.[7, 18] However, those studies involved populations subject to different genetic and environmental factors from ours. It was of interest, therefore, to investigate in our country the prevalence of ANA, to examine the difference in staining patterns among patients with various connective tissue diseases, and to compare our results with those of other reports.

MATERIALS AND METHODS

Population studied

Sera from 335 patients diagnosed with various connective tissue diseases and sera from 43 normal healthy controls were tested for the presence of ANA by indirect immunofluorescent technique. The distribution of patients for each disorder is tabulated in Table 1. All patients with diagnoses of SLE, RA, and scleroderma (PSS) fulfilled the ARA criteria for diagnosis of these diseases.[19] The patients grouped under overlapping syndromes were those who demonstrated features of more than one connective tissue disease. Among this group, 12 had mixed connective tissue disease,[11] and 5 had features suggestive of sclerodermatomyositis without high titer of anti n-RNP. The remainder were non-classified.

Table 1 Distribution of patients among the different connective tissue diseases.

Diseases	Number
Systemic lupus erythematosus (SLE)	135
Rheumatoid arthritis (RA)	123
Scleroderma (PSS)	52
Overlapping syndromes* (OS)	25
Normal controls	43

* Overlapping syndromes – Patients who manifested the features of more than one connective tissue diseases.

Indirect immunofluorescent test

The technique for ANA determination by indirect immunofluorescence was employed with 5 μ cryostat sections of mouse kidney as the substarte. The details of the procedures have been previously described.[20] A polyspecific sheep antiserum to human immunoglobulin conjugated with fluorescein isothiocyanate was purchased from Welcome Reagents Limited, London, England. The pattern and intensity of fluorescence were evaluated at 400 X with an Olympus reflected light fluorescence microscope with a 200W mercury light source, an Ey 455 exciter filter and a 0530 barrier filter. Tests were carried out using the four serum dilutions of 1:4, 1:16, 1:64 and 1:256. Sera giving 1+ or greater fluorescence at a dilution of 1:16 were considered positive.

It is possible that more than one pattern of ANA could be observed in a particular rheumatic disease, for example, in SLE, all patterns were described in one serum. However, in other diseases as MCTD the pattern was characterized mainly by the speckled pattern. For reason of simplicity, we decided to report on the incidence of individual patterns observed.

RESULTS

Prevalence of ANA in different connective tissue diseases

Figure 2 depicts our findings on ANA positivity among patients with different connective tissue diseases and among the controls. In this study, the frequency of ANA among 43 control sera was found to be 11.6 percent and this is comparable to the frequency described in the study[21] using the same technique and substrate. Although prior study in normal Thai adults[22] showed lower percentage of seropositivity, the dissimilarity could be due to the difference in substrate and fixatives utilized. The frequency of ANA positivity among our controls was significantly different from each group of the diseased populations ($P<0.01$). The only pattern observed among normals was the speckled pattern at a low titer. Among the disease groups, percent positivity of sera from patients with SLE was 97.0 percent, with RA 31.7 percent, with scleroderma (PSS) 90.4 percent and with overlapping syndromes 80.0 percent. The frequencies of positive ANA sera among the disease groups are comparable to those reported in the literature.[7, 18, 23, 24]

ANA patterns in SLE

As demonstrated in Figure 3, the most common pattern of ANA in patients with SLE observed in this study was speckled (67.9 percent of ANA positive sera). The rim pattern was noted in 58.0 percent and the homogeneous pattern in 52.7 percent.

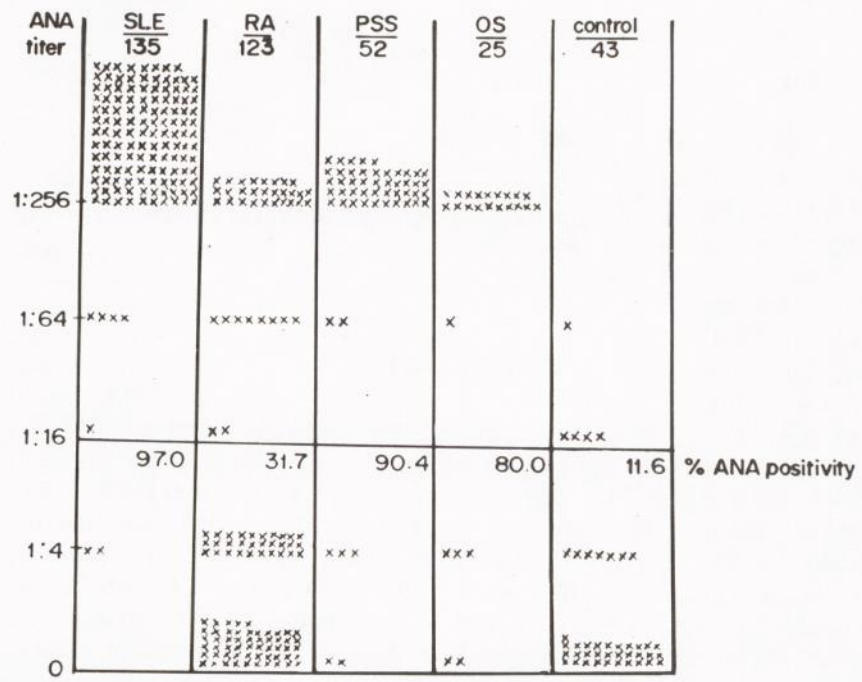

Fig. 2 Antinuclear antibodies in different connective tissue diseases and controls.

ANA patterns in RA

Figure 4 shows the distribution of ANA patterns produced by sera from RA patients in this study. Of the 39 ANA positive sera, it was found that only 15.4 percent showed the homogeneous pattern while 76.9 percent exhibited the speckled pattern. The rim pattern was shown in 5.1 percent and the nucleolar pattern in 2.6 percent.

ANA patterns in scleroderma

Serum from patients with scleroderma may produce a variety of ANA patterns. However, with the use of mouse kidney as a substrate, some patterns may be obscured.[14] The only two ANA patterns observed in scleroderma utilizing mouse kidney were speckled and nucleolar.[14] We observed the same phenomenon among sera from our patients (Fig. 5). Although the frequency of the nucleolar pattern was reported to be as high as 54 percent among scleroderma sera in Ritchi's study,[23] it was only 17 percent in our study.

ANA patterns in overlapping syndromes

Although overlapping syndromes are less defined clinical entities, two of them have been singled out as distinct disorders: mixed connective tissue disease (MCTD), a disorder with anti-n-RNP in high titer as its marker,[11] and sclerodermatomyositis with anti-Ku.[25] On ANA testing, most of the disorders in these overlapping syndromes demonstrate the speckled pattern of nuclear staining in common.[11] However, other patterns may occasionally be described[25] depending upon the connective tissue diseases that constitute the syndrome. In the present study, the speckled pattern was found in 90 percent of the ANA positive sera and the rim pattern in 10 percent (Fig. 6).

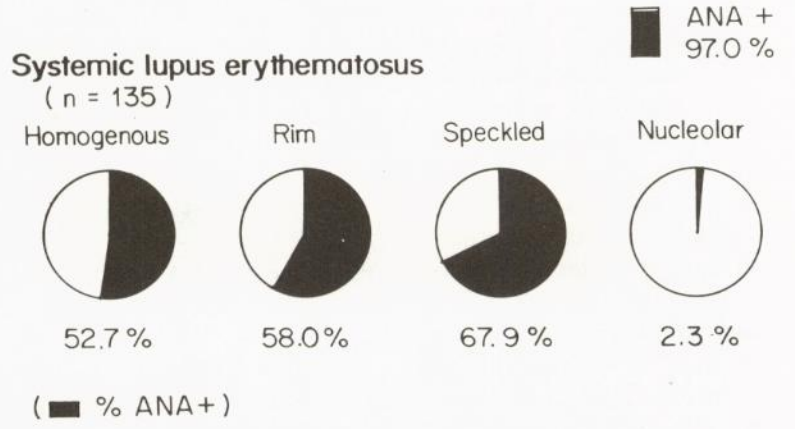

Fig. 3 Distribution of ANA patterns in ANA-positive patients with systemic lupus erythematosus.

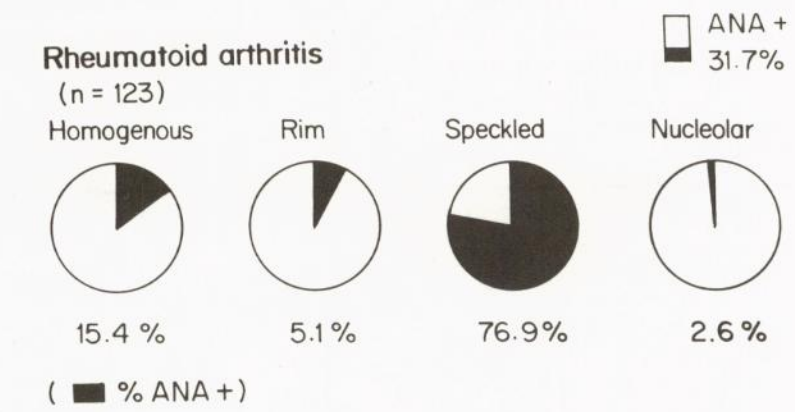

Fig. 4 Distribution of ANA patterns in ANA-positive patients with rheumatoid arthritis.

DISCUSSION

The results of this study are not unlike those reported previously [7,18,23,24] on the prevalence of ANA in different connective tissue diseases. However, some discrepancies were observed with regard to the frequency of patterns detected in certain diseases.

In SLE, prior investigators have reported that the most frequently observed pattern of ANA is the homogeneous pattern (59-67 percent of ANA positive sera).[7,18] The frequency of the rim pattern was reported to be 39 percent[7] and the speckled 2-30 percent.[7,18] In contrast, the present study found that the most frequent pattern for SLE patients was speckled (67.9 percent) at a much higher frequency than previously reported. The homogeneous pattern was noted at an even lower frequency than the rim pattern (52.7 percent and 58.0 percent respectively). A discrepancy

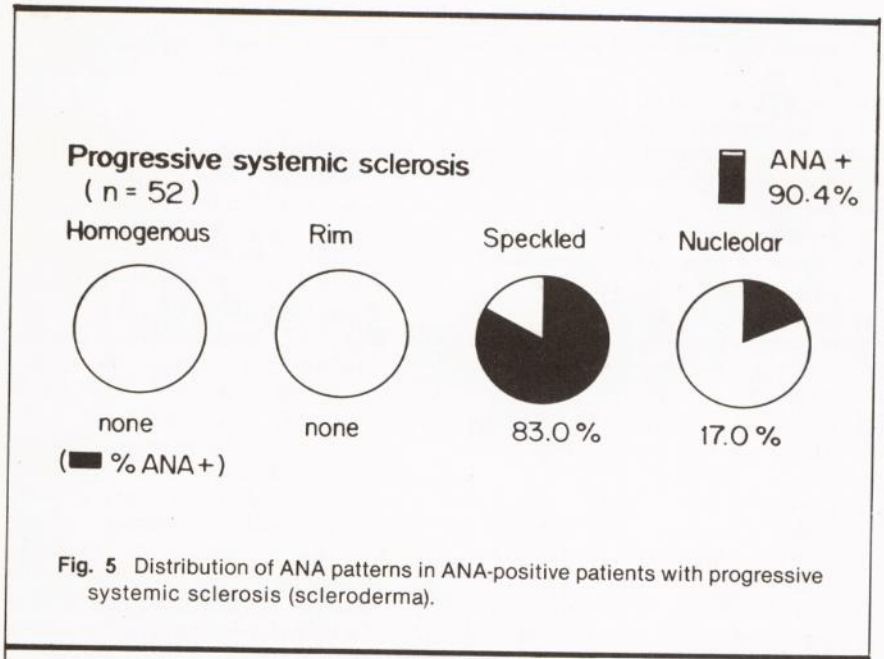

Fig. 5 Distribution of ANA patterns in ANA-positive patients with progressive systemic sclerosis (scleroderma).

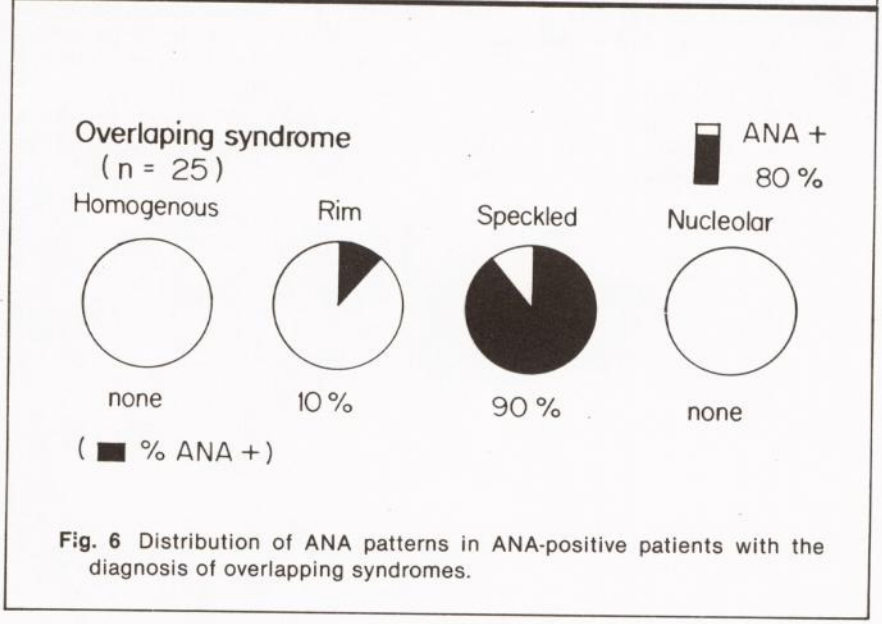

Fig. 6 Distribution of ANA patterns in ANA-positive patients with the diagnosis of overlapping syndromes.

was also found in the ANA patterns observed among patients with RA. Prior studies[7,18,21] on ANA in RA patients suggested that antihistone antibodies which produced the homogeneous ANA pattern were frequently found. However, we observed that the speckled pattern; was five times more frequent than the homogeneous pattern.

The reason for these discrepancies in unclear. Possible explanations may firstly include differences in the genetic influences among the populations studied. It has been suggested that HLA patterns among the Thai may differ from those found elsewhere.[26] Secondly, environmental factors may contribute to variations in the expression of the diseases; therefore, the difference in ANA patterns observed in patients in this study may reflect differences in the manifestation of the diseases seen here as compared with elsewhere. Until the pathogenesis of these connective tissue diseases is understood, these may be the only explanations.

In scleroderma, we observed a lower frequency of the nucleolar pattern compared with an earlier group.[23] This may, in part, be attributed to the difference in the substrate used for detecting the nucleolar antigen. However, considering the sensitivity of the test utilized by our study, it may be possible that patients with scleroderma may actually have expressed different ANA specificity.

Further study on the correlation of serologic abnormalities and clinical features among patients with this group of diseases in Thailand may contribute to a better understanding of the variation in serologic manifestations among patients with these disorders.

ACKNOWLEDGEMENTS

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