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The Autoantibody Profile and Its Association with Clinical Manifestations in Malay SLE Patients

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SUMMARY **A cross sectional study was conducted to determine the autoantibody profile of Malay SLE patients in Kelantan, North East Malaysia and to correlate them with clinical presentations. Eighty-two Malay SLE patients who fulfilled the ARA criteria underwent the following tests: ANA, anti-dsDNA antibody, anti-ENA antibody and RF. The results revealed that ANA was positive in 91.5% of the patients, anti-dsDNA antibody in 53.7%, however, anti-ENA antibodies were positive in only 9.8% of the cases at the time of the study and none had a positive RF. The profile of autoantibodies was similar to other studies except for a lower incidence of anti-ENA antibodies. Sixty three percent of patients had lupus nephritis. The pattern of clinical presentations were noted to be more similar to those found among Chinese and Indian SLE populations than compared to the Caucasians. There was a significant association between anti-dsDNA antibody and lupus nephritis and between anti-ENA antibody and thrombocytopenia.**

Systemic Lupus Erythematosus (SLE) is a chronic disease which is characterized by a breakdown of immunological tolerance, production of autoantibodies and inflammation in multiple organs leading to a wide variety of clinical manifestations.[1] The incidence and prevalence varies according to ethnic background, sex and age.[1] A study by Hopkinson *et al.*[2] showed that the highest prevalence was seen in Afro-Carribeans, followed by Asians and Caucasians. The etiology of SLE remains unknown. A genetic predisposition, sex hormones and environmental factors do play an important role in the pathogenesis of SLE.[1]

Studies have been done in different populations such as in Caucasians, in Turks, in Blacks, in Indians and also in Chinese populations regarding antibody profile and clinical presentation of SLE patients. However, no such study has been done in the Malay population.

A person is suspected to have SLE if she/he fulfils 4 out of 11 criteria based on the America Rheumatism Association's 1987 criteria (revised criteria for the classification of systemic lupus erythematosus).[4] A combination of anti-dsDNA, C3, C4, C-Reactive Protein (CRP) and erythrocyte sedimentation rate (ESR) assays provides the most useful information for diagnosing SLE.[5]

The most frequent presentation found in Caucasians and Chinese Singaporean was involvement of skin and joints[3,6] whereas nephropathy and malar rash were more common in the Chinese population in Hong Kong.[7] Other systems that can be involved in SLE are blood, kidney, brain, eye, lung, heart and the gastrointestinal tract.

The main types of antibodies

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that can be found in SLE patients are anti-nuclear antibody (ANA), anti-dsDNA (deoxynucleic acid) antibody and anti-extractable nuclear antigen (ENA) antibody. Anti-ENA antibodies include anti-Smith (Sm), anti-ribonucleoprotein (RNP), anti-Ro and anti-La antibodies.[1]

ANA was detected in 91% of SLE patients in a Turkish population,[8] in 98% of Caucasians SLE patients[3] and was invariably positive in the Chinese population from Hong Kong.[7] Anti-ENA antibodies were found to be positive in varying degrees in the Chinese population from Hong Kong whereby anti-Ro antibodies were found in 60.2% of their SLE patients.[7] However, only 27% of a Turkish population had anti-Ro antibodies.[8] In Caucasians, anti-RNP antibodies were more common than anti-Ro antibodies (40%).[3] Other types of autoantibodies that can be found in SLE patients are ribosomal P-protein antibodies, antibodies to proliferating cell nuclear antigen (PCNA), rheumatoid factor (RF), anti-phospholipid antibodies, anti-platelet antibodies, anti-red blood cell antibodies and anti-leucocyte antibodies.

The aims of this study were firstly to determine the frequency of various auto-antibodies that can be found in Malay SLE patients in Kelantan, Malaysia, using indirect immunofluorescence and immunoprecipitation techniques; secondly to ascertain the clinical manifestations that can be found in our SLE patients and to determine whether there is any association between the type of autoantibodies detected and the clinical presentations.

MATERIALS AND METHODS

Patients

A cross sectional study was conducted between December 1999 to November 2000 to determine the frequency of several autoantibodies and to correlate them with clinical presentations in Malay SLE patients attending the Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. Eighty-two Malay SLE patients who were diagnosed based on the revised criteria for the classification of systemic lupus erythematosus were included in this study. Excluded were those SLE patients who were either in the pediatric age group (below 13 years old) or had drug induced lupus.

Each of the subjects underwent the following tests: ANA, anti-dsDNA antibody, anti-ENA antibody and RF. Clinical data were recorded on a standard form.

ANA and anti-dsDNA measurements

Anti-nuclear antibody and anti-dsDNA antibody were performed using an indirect immunofluorescence technique (Biosystem, Barcelona, Spain) on Hep-2 cells and *Crithidia luciliae* as substrates, respectively.

ENA antibody measurement

Extractable nuclear antigen (anti-Sm, anti-RNP, anti-Ro and anti-La) analyses were performed using the double immunodiffusion (Ouchterlony) technique (Scimedx, New Jersey, USA).

RF measurement

RF was detected using latex agglutination technique (Humatex RF, Wiesbaden, Germany).

Statistical methods

Data were analyzed using the SPSS version 10 software.[9] Frequency of autoantibody detection and clinical manifestations were reported in percentage over total sample size (82 subjects). The association between autoantibody presence and clinical manifestations was tested using Simple Logistic Regression and was reported as Pearson correlation (r) and odds ratio (OR). A p value of less than 0.05 was considered as significant at a 95% confidence interval (95% CI) for the correlation.

RESULTS

Eighty-two Malay SLE patients with a median age of 28.5 years (interquartile range of 18.25) were enrolled in this study. The age was presented as median ± interquartile range due to non-normal distribution. There were 73 (89%) females and 9 (11%) males in the study.

Anti-nuclear antibodies (ANA) were positive in 75 (91.5%) SLE patients. However, according to a review of the medical records, all the patients were ANA positive at the time of diagnosis. Out of these patients, 49 (65.3%) showed a homogeneous pattern and 26 (34.7%) showed a speckled pattern. Thus there were 7 patients with negative ANA at the time of the study but who had been positive at the time of diagnosis. Anti-dsDNA antibodies were found to be positive in 44 patients (53.7%). ENA antibodies were positive in 8 patients (9.8%) whereby they were 2

(25%) patients with anti-Sm antibody, 3 (37.5%) with anti-RNP antibody, 2 (25%) with both anti-Sm/RNP antibodies and 1 (12.55%) patient with anti-La antibody. None of the patients were positive for rheumatoid factor. The various autoantibodies found in the patients are shown in Table 1.

Homogeneous ANA showed a significant association with anti-dsDNA antibodies ($p < 0.001$, r = 0.61) and speckled ANA was significantly associated with ENA antibodies ($p = 0.01$, r = 0.31). Table 2 shows the comparison of the autoantibody profile of this study to other series.

The most prominent clinical presentation in the patients was arthritis and/or arthralgia (69.5%). About 52 patients (63.4%) had lupus nephritis based on clinical manifestations, urine microscopy or renal biopsy. Twenty-nine (55.7%) of the patients that underwent renal biopsy had type IV nephritis according to the WHO classification. The most prominent hematological disorder was normochromic normocytic anemia (23.2%). Malar rash was seen in 35 patients (42.7%) and lupus cerebritis in 16 patients (19.5%). Ocular, cardiac and pulmonary manifestations occurred less commonly. None of the patients had any gastrointestinal manifestations due to SLE. The occurrence of different clinical manifestations is shown in Table 3. Among 9 (11%) thrombocytopenic patients, only 1 (1.2%) was on cyclophosphamide therapy.

Simple logistic regression tests showed that lupus nephritis SLE patients had 4.8 times the chance of having positive anti-dsDNA antibodies results (OR: 4.8 [95% CI: 1.82-12.7], $p = 0.002$) and thrombocytopenic SLE patients had 6.8 times the chance of having positive ENA antibodies results (OR: 6.8 [95% CI: 1.3-35.6], $p = 0.02$).

Table 1 Autoantibodies found in 82 Malay SLE patients

Autoantibodies	Number (n)	Percentage (%)
ANA	75	91.5
Anti-dsDNA	44	53.7
Anti-RNP	5	6.1
Anti-Sm	4	4.9
Anti-La	1	1.2
Anti-Ro	0	0
RF	0	0

Table 2 Comparison of autoantibodies in Malay, Turkish, Chinese and Caucasian SLE patients

Autoantibodies	Malay (2000) %	Turkish (1997) %	Chinese (1993) %	Caucasian (1998) %
ANA	91.5	91	High	98
Ds-DNA	53.7	64	NA	70
Anti-RNP	6.1	NA	28.8	40
Anti-Sm	4.9	7	12.7	30
Anti-La	1.2	7	8.4	10
Anti-Ro	0	27	60.2	30
RF	0	30.3	NA	30

NA, Not available

Table 3 Clinical manifestations of 82 Malay SLE patients

Manifestation	Number	%
Arthritis/arthralgia	57	69.5
Lupus nephritis	52	63.4
Skin and mucous membrane	43	52.4
Normochromic normocytic anemia	19	23.2
Lupus cerebritis	16	19.5
Hemolytic anemia	16	19.5
Ocular	14	17.1
Thrombocytopenia	9	11
Leucopenia	8	9.8
Cardiac	6	7.3
Pulmonary	4	4.9
Myopathy/myositis	3	3.7

Table 4 Clinical manifestations seen in SLE patients among Malaysian Malays, Singaporean Chinese, Sri Lankan Indians and the American Caucasians

Clinical Manifestation	Malay (Malaysia) 2000 %	Indian (Sri Lanka) 2000 %	Chinese (Singapore) 1992 %	Caucasian (America) 1998 %
Arthritis/arthralgia	69.5	44	44	60
Lupus mephritis	63.4	69	NA	50
Skin and mucous membrane	52.4	98	52	80
Lupus cerebritis	19.5	42	4	60
Vasculitis	21.3	78	NA	NA
Hemolytic anemia	19.5	54	3	10
Thrombocytopenia	11	54	4	15
Ocular	17.1	NA	NA	15
Cardiac	7.3	16	3	NA
Pulmonary	4.9	10	7	60
Gastrointestinal	NA	NA	7	45

NA, not available

DISCUSSION

This was the first study comparing the autoantibody profile and clinical manifestations among Malay SLE population. As in most other ethnic groups, the ANA positivity was very high.[3,7,8] All patients were ANA-positive upon diagnosis and 91.5% were still positive at the time of this study. A positive ANA test is not specific for SLE as ANA occurs in some normal individuals (in low titer) and the occurrence increases with age.[3]

Antibodies to dsDNA and to Sm are relatively specific for SLE.[3] High serum levels of ANA and anti-dsDNA antibodies together with low levels of complement usually reflect disease activity especially in patients with nephritis.[3] The anti-dsDNA antibody was positive in 53.7% of cases at the time of the study. This was comparable with other studies done in Caucasians,[1,3] the Turkish population[8] and the Hong Kong Chinese population.[7] However, many patients in this study were on treatment and the percentage of patients with anti-dsDNA antibodies at diagnosis might have been higher since some may have become negative with treatment.

Most SLE patients have immunoglobulins deposited in glomeruli, but only one-half have clinical nephritis, defined by proteinuria and microscopic hematuria with urinary casts.[3] Lupus nephritis was a very common clinical manifestation in the Malay population, comparable to the Sri Lankan population (69%)[10] and the Hong Kong Chinese population (69.3%).[7] There was a strong correlation between anti-dsDNA antibody and lupus nephritis which was consistent with the findings of Warlow.[11]

ENA antibodies were uncommonly seen in this study population compared to other studies.[3,7] This may be related to the high incidence of SLE nephritis, which is usually associated with a negative ENA antibody status. Tapanes *et al.*[12] found that the absence of anti-ENA antibodies increased the odds ratio to develop SLE nephropathy eleven fold. Anti-ENA antibodies were associated with thrombocytopenia, also similar to the findings by Warlow.[11] None of our patients were positive for RF. Howard *et al.*[13] stated that patients with SLE who are negative are likely to develop renal disease whereas those who are RF positive are very unlikely to do so. In our study, a similar trend was observed since the majority of the patients had renal problems and a negative RF. The most common major organ involvements found in this study were renal (63%) and hematological (63%) followed by neurological disorders (19.5%). This was almost similar

among the Chinese and Indian populations.[6,7,9] Only one out of nine patients with thrombocytopenia was on cyclophosphamide therapy. It is difficult to determine whether the thrombocytopenia was related to the drug therapy or the active disease. Among the Caucasians, pulmonary lupus and lupus cerebritis were more common. The comparison of clinical manifestations between four different ethnic groups is illustrated in Table 4.

In conclusion, the antibody profile among the Malay population with SLE was comparable with that found in other populations except for a lower incidence of ENA antibody. Regarding the clinical manifestations, there were some differences in the percentage of involvement of various organs compared to other ethnic groups studied in other parts of the world. However, there were some similarities found with Chinese and Indian SLE patients. The associations between anti-dsDNA antibody and nephritis and between anti-ENA antibody and thrombocytopenia, found in other studies were similarly noted in this study.

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