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The Prevalence of Antinuclear, Anti-dsDNA, Anti-Sm and Anti-RNP Antibodies in a Group of Healthy Blood Donors

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SUMMARY **We studied the prevalence of antinuclear (ANA), anti-double stranded DNA (dsDNA), anti-Sm and anti-RNP antibodies in a group of 93 blood donors (age range: 18-58 years). Antinuclear and anti-ds DNA antibodies were detected by immunofluorescence (IF) using HEp2 cells and *Crithidia luciliae* as substrates, respectively, while anti-Sm and anti-RNP antibodies were assayed by ELISA. ANA was found in 6.5% while anti-dsDNA antibodies were not detected in any of the subjects. The 98th percentile was used as cut off where values greater than 0.651 for anti-Sm and 0.601 for anti-RNP antibodies were taken to be positive. This gives a frequency of 1.1 % for both antibodies. There was no significant association of antibody positivity with sex or race. We conclude that certain autoantibodies are present in low titres in the normal Malaysian individuals, at a different frequency compared to other studies probably due to genetic,ethic or environmental factors.**

Autoimmune diseases are characterized by the presence of serum autoantibodies which damage body organs. Antibodies to nuclear antigens are used as markers for some clinical subsets of SLE. Anti-dsDNA antibodies are markers of diagnostic and prognostic significance. Anti-Sm is a highly specific marker for SLE.[2] Anti-RNP and anti-Sm antibodies usually exist together in patients with SLE but a high titre of anti-RNP are found exclusively in patients with mixed connective tissue disease.[3]

However, autoantibodies can occur in normal healthy individuals.[4-8] In some individuals several autoantibodies can occur simultaneously. This present study was undertaken to determine the prevalence of these autoantibodies in a population of healthy Malaysian blood donors and to determine the association among ANA, anti-dsDNA, anti-Sm and anti-RNP antibodies. Prevalence data are also useful for determining reference point for laboratory specimens.

MATERIALS AND METHODS

Serum samples were obtained from 93 blood donors (70 Malays and 23 Chinese, age range: 18 to 58 years) attending the Blood Bank Service of Hospital Kuala Trengganu in early 1995.

Detection of ANA and anti-ds DNA antibodies

ANA were detected by immofluorescence (IF) using HEp-2 cells as substrate (INOVA Diagnostics, San Diego, CA). Staining was done with

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fluorescein-conjugated goat anti-human polyvalent immunoglobulin (Behringwerke, Marburg, Germany). Nuclear fluorescence at a serum dilution of 1:40 or higher was considered as positive. Anti-ds DNA antibodies were performed by IF employing *Crithidia luciliae* as substrate. A titre of 1:10 or higher was regarded as positive. Both positive and negative controls were included.

Table 1: Distribution of blood donors with positive ANA. (C=Chinese; M=Malay)

Sex	Race	Age	ANA	Pattern
F	C	18	1:40	Speckled
F	C	30	1:40	Nucleolar
M	C	31	1:40	Speckled
M	M	35	1:40	Speckled
M	M	39	1:40	Speckled
M	C	38	1:80	Homogeneous

Detection of anti-Sm and anti-RNP antibodies

These antibodies were assayed using an ELISA kit (INOVA Diagnostics, San Diego, CA) with microtiter wells precoated with affinity purified Sm and Sm-RNP antigens. Conjugate used was goat anti-human IgG. Plates were read at absorbance 450 nm and results recorded in OD units.

Statistical analysis

Data were entered and analysed with SPSS. Frequency distribution patterns for both anti-Sm and anti-RNP antibodies showed skewed patterns and a non-parametric analysis was used. The 98th percentile was taken as the cut-off point. Association among autoantibody positivities was determined using a x2 analysis or Fisher's exact test where appropriate. A p value of < 0.05 was taken to be significant.

RESULTS

Positivity for ANA among our study group was 6.0% at a titre of 1:40 showing three different kinds of staining patterns. Only one individual remained positive at 1:80 (Table 1.) Raised titres were found in 2/19 (10.5%) females and 4/74 (5.4%) males ($p > 0.05$). The Chinese race was found to have a higher prevalence of ANA ($p<0.05$). When anti-ds DNA antibody test was performed we did not find any positive case with a starting dilution of 1:10.

The frequency distribution patterns are shown in Figs. 1 and 2 for anti-Sm and anti-RNP antibodies, respectively. Employing the 98th percentile, OD readings of above 0.651 for anti-Sm and 0.601 for anti-RNP antibodies were taken to be positive thus giving a prevalence of 1.1%. Only one subject each showed raised levels of anti-Sm and anti-RNP antibodies.

Antibody association

No association was found among the different antibodies detected ($p>0.05$).

DISCUSSION

The Autoantibodies occur in healthy individuals but their significance is unclear. Several studies have looked into the prevalence of ANA in healthy individuals but information is lacking on the prevalence of anti-Sm and anti-RNP antibodies. This study was undertaken to study the prevalence of these antibodies in healthy Malaysian blood donors and their possible association. Immunofluorescence has been the standard technique for the detection of ANA. For the specific detection of anti-Sm and anti-RNP antibodies the technique of double diffusion (DD) is most commonly used.[9] Later, the CIE was used but recently the ELISA using purified antigens has been more frequently used for convenience as well as sensitivity[10] and specificity.

ANA occurred in 6.5% of 93 blood donors using HEp2 cells as substrate and speckled, homogeneous and nucleolar patterns. ANAs are known to occur more commonly among females.[11-13] In our study we observed a higher frequency of ANA among females, the association was not significant. Prevalence was higher in the Chinese individuals. The prevalence of ANA among 582 normal subjects using rat kidey and stomach sections as substrates was 4.8%.[11] A separate study on 107 blood donors showed a prevalence of 4.5% at a titre of at least 1:10 using mouse liver, kidney and stomach substrate.[14] However, a 2% frequency of ANA was found in 466 blood donors at 1:16 or higher using human thyroid as substrate,[15] while the ANA frequency of 2,500 female blood donors demonstrated a rate of 15.9% at titre of > 1:20 and 1.1% at 1:80 using HEp2 cell substrate. The occurrence of ANA in 485 healthy blood donors was found to be 12.8% with HEp2 cells at a titre lower than or equal to 1:80.[16] However, in a group of 664 healthy Saudi individuals the prevalence of ANA was 4.2%.[17] Discrepancies in prevalence rates may be due to genetic, ethnic or environmental factors.[18] Furthermore, the study groups differ in age, sample size, sex ratio and methods of antibody detection.

The prevalence of specific autoantibodies in our local normal population has not been previously

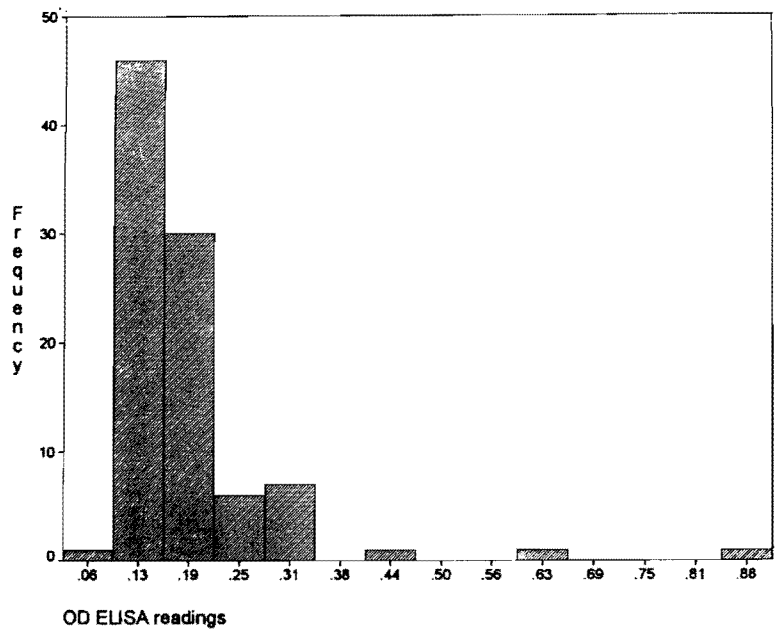

Fig. 1. Frequency distribution of anti-Sm antibodies in 93 blood donors.

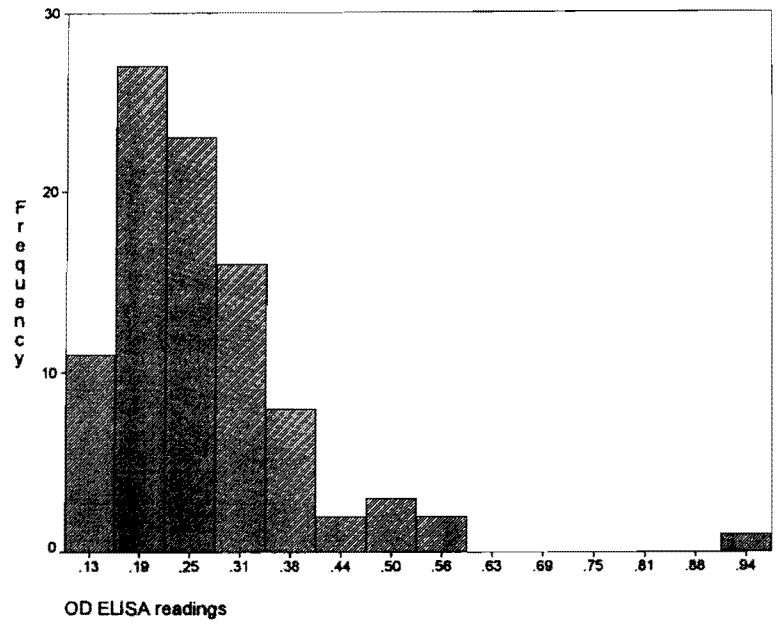

Fig. 2. Frequency distribution of anti-RNP antibodies in 93 blood donors.

investigated. In this study we observed a prevalence of 1.1% for both anti-Sm and anti-RNP antibodies. Anti-Sm and anti-RNP antigens have been found to be closely related immunologically.[2] In the RNP ELISA, microtiter plates are coated with an RNP/Sm antigen complex. When reporting results, it is advised by the manufacturer that the patient's Sm antibody reponse should be taken into account. With positive Sm/RNP results, the anti-Sm activity has to be assessed in order to make this distinction. If a sample is negative in the Sm ELISA but positive in the RNP ELISA, then the activity is largely due to RNP antibody. If, however, the activity of Sm ELISA is equivalent to the activity of the RNP, then the sample has primarily anti-Sm antibody.

Certain infections[19] and medications may also cause autoantibody production[20] in normal individuals. Autoantibody may also be found in healthy relatives of patients with connective tissue diseases[21] as well as individuals in a subclinical autoimmune state. We conclude that autoantibodies occur in normal individuals in low titres and their presence may not signify an autoimmune state.

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