

Anti-nuclear, anti-mitochondrial, anti-smooth muscle and anti-parietal cell antibodies in the healthy Malaysian population

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Abstract

A study of 101 sera from 69 Malay, 14 Chinese and 18 Indian healthy adult Malaysians was undertaken to determine the frequency of antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), antismooth muscle antibodies (SMA) and antiparietal cell antibodies (APCA). There were 67 females and 34 males with a mean age of 31.7 years (+/-8.6). ANA was assayed by immunofluorescence (IF) using both mouse liver and HEp-2 cell substrates. AMA, SMA and APCA were also tested by IF using composite sections from mouse liver, kidney and stomach substrates. Analysis showed 6.9% were positive for ANA at a titre of 1:40 with HEp-2 while only 1.9% were detected using mouse liver. 9.9% had detectable AMA from titres 1:10 to 1:90. None of them had detectable SMA and only 1 (0.09%) had APCA at a titre of 1:80. This study suggests that a diagnosis of an autoimmune disorder has to be cautiously made taking into consideration that autoantibodies are present in low titres in the healthy population.

Key words: Antinuclear antibody, antimitochondrial antibody, antismooth muscle antibody, antiparietal cell antibody, autoantibodies, healthy population.

INTRODUCTION

Autoimmune disorders (AID) develop when there is a breakdown of tolerance to self-antigens giving rise to the production of serum autoantibodies directed against a variety of cellular and extracellular antigens. The detection of these autoantibodies is an important tool for the diagnosis of these disorders,¹ but its absence does not rule out the presence of AID. Antinuclear antibodies (ANA) are closely associated with systemic rheumatic diseases but are also found in healthy subjects in low titres.²

The increasing awareness among clinicians from the many requests for autoantibody screening in the past few years and the difficulty in interpreting low titre results has prompted us to carry out this study on the frequency of various autoantibodies in the normal healthy Malaysian population. These seropositive asymptomatic individuals may be healthy first degree relatives of patients with AID, have certain genetic predisposition destined to develop AID in later life or merely have them as a result of chronic infection or inflammatory states.

The use of various tissue substrates in the clinical laboratory forms the basis of the assays that detect autoantibodies. In the 1970s, cryostat sections of rodent liver or kidney were widely used, but are now replaced by the more sensitive,

easily available HEp-2 tissue culture cell substrate, a continuous cell line of human laryngeal carcinoma. The latter-mentioned substrate is at an advantage in detecting certain autoantibodies not detected by the conventional cryocut sections. Because of this shift towards the increased usage of HEp-2 cells, clinicians will face interpretative problems unless the prevalence of ANA in the normal population is studied and a cut-off titre established.

MATERIALS AND METHODS

One hundred and one sera samples were obtained from normal individuals, comprising hospital staff, factory workers and students of the School of Medical Laboratory Technologists at the Institute for Medical Research (IMR), Kuala Lumpur. The individuals had no symptoms or signs of autoimmunity, any immediate family history of autoimmunity or any chronic infections. All sera were stored at -20°C until performance of assays.

Serum samples were assayed for the presence of ANA, anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (SMA) and antiparietal cell antibodies (APCA) using the standard immunofluorescence (IF) technique. ANA was determined using both cryostat sections of mouse liver and HEp-2 cell substrate

(Immunoconcept, Sacramento, Ca, USA). An initial overlay of test sera was started at 1:10 dilution with PBS pH7.2 followed by fluoresceinated polyvalent rabbit anti-human immunoglobulin (Behringwerke, Marburg, W.Germany) with a F/P ratio of 2.5+/-1.5. Screening dilution using HEp-2 cell was carried out at 1:40 as recommended. Positive sera was repeated with doubling dilutions. The intensity of fluorescence was read with a Leitz Ortholux II incident light microscope. Results were taken to be positive when the intensity of immunofluorescence was graded at greater than 1+ using a scale that ranged from 0 (no fluorescence) to 4+ (highest intensity fluorescence).

Presence of AMA, SMA, APCA were also determined by IF using cryocut composite sections of mouse liver, kidney and stomach tissue. Further doubling dilutions were also performed on positive specimens.

RESULTS

The subjects comprised of 67 females and 34 males (69 Malays, 18 Indians and 14 Chinese) with a mean age of 31.7 years (+/- 8.6 years) with ages ranging from 20 to 54 years. The sex distribution of seropositive cases is shown in Fig. 1. There was no significant sex difference in subjects with ANA. Females had a higher

TABLE 1: Frequency of autoantibodies in 101 normal individuals

Autoantibodies	Positive (n)	Percentage
ANA (Mouse liver)		
1:10	9	8.9
1:20	3	2.9
1:40	2	1.9
ANA (HEp-2)		
>= 1:40	7	6.9
AMA		
1:10	3	2.9
1:20	2	1.9
1:40	5	4.9
SMA		
1:10	0	0
APC		
1:10	1	0.9

frequency of AMA (11.9% vs. 5.9%). None of the subjects had SMA and only one had APCA.

Individuals who were positive at the initial screening dilution was further tested at two-fold dilutions and results recorded as in Table 1. 14 (13.9%) were positive for ANA with mouse liver substrate and 7 (6.9%) with HEp-2 cells.

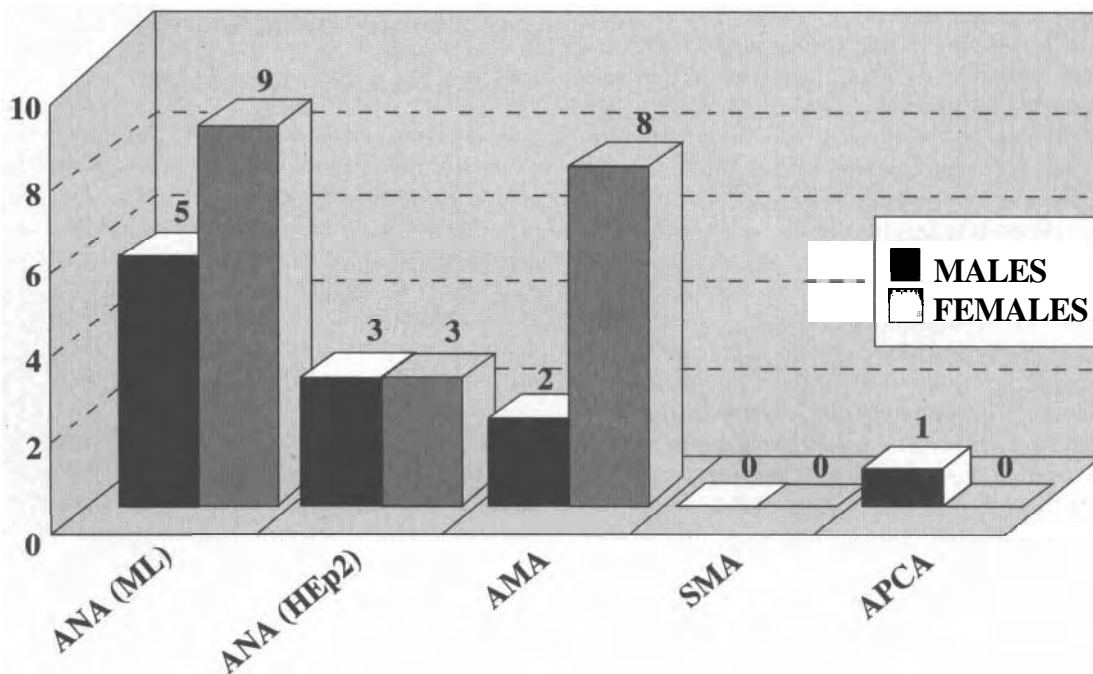


FIG 1: Sex distribution of seropositive individuals

AMA was detected in 10 (9.9%) individuals at dilutions equal to or greater than 1:10. SMA was not detected. As for APCA, only one (0.9%) was found to be positive at a titre of 1:80.

DISCUSSION

The presence of ANA in serum is associated with the diagnosis of AID.' Interpretation of laboratory tests for autoantibody screening has always been a dilemma for clinicians because they can also be found in the healthy population though at low titres. Their presence is usually detected either by screening the healthy population or finding them accidentally when using them as healthy controls for patients with AID. Laboratory autoantibody testing can only be used as an aid in reaching a diagnosis. The clinical manifestations of the patients have always to be taken into consideration.

The gold standard for a number of autoantibody screening methods has been the immunofluorescence method. Several types of substrates can be used, and they differ in their ability to detect certain autoantibodies. Our observation that 7 of the study population had a positive ANA on HEp-2 at a titre of 1:40 compared to only 2 using mouse liver substrate supports HEp-2 cells as being more sensitive than cryocut sections. The initial dilution of 1:40 with HEp-2 cells was recommended because of its increased sensitivity.³

We detected 14 (13.9%) with ANA (mouse liver) at a dilution of equal or greater than 1:10, where 9 (8.9%) had a dilution of 1:10, 3 (2.9%) at 1:20 and 2 (1.9%) at 1:40. Using HEp-2 cells, 7 (6.9%) had ANA titre at equal to or greater than 1:40. A study on 1242 healthy Malaysians using different types of substrates demonstrated a frequency of 2% and 7.2% with mouse liver at a cut-off titre of 1:10 and HEp-2 cells at 1:20 respectively.⁴ A recent study conducted among 290 healthy Swedish adults, demonstrated a prevalence rate of 6.9% and 6.0% using HEp2 and mouse liver substrate respectively at a titre of 1:40.⁵ Another study revealed a frequency of 4.8%⁶ at a titre of 1:10. Screening for ANA on female blood donors showed a prevalence rate of 15.9% and approximately 4% at titres greater than 1:20 with HEp-2 cells and mouse liver sections respectively.³

AMA is not exclusively found in patients with primary biliary cirrhosis and the significance of their presence in the normal population is unknown.⁷ Studies on the frequency of AMA have recorded a range of 0.3-1.64%.⁸ We have

found 10/101 individuals (9.9%) with AMA, 3 at a dilution of 1:10, 2 at 1:20 and 5 at 1:40, where 11.9% were females and 5.9% males. However, in a study on female blood donors a 3.6% frequency was observed at a titre of 1:20, 5% at a titre of 1:40 and 1% at 1:80.³

SMA have been detected in healthy individuals. The incidence of SMA in the normal population has been reported to be in the range of 1.5-7.5%.^{9,10} Higher frequencies of 11.3-20% were reported elsewhere.^{11,12} A titre of 1:20 or less was taken as being positive. In our study we did not detect any SMA. Our failure of getting a positive case here could be due to a smaller sample population as compared to other studies. Furthermore, positivity might also vary depending on the type of conjugate used, the microscope employed and interobserver variability.

Only 1 (0.9%) subject had detectable APCA at a dilution of 1:80. This was found in a 21-year-old Malay male who also had concomitant ANA (1:40) and AMA (1:40) but was asymptomatic. A frequency of 7.5% was seen in a study screening for autoantibodies among blood donors at a titre of 1:10 to 1:80.¹⁰ Variability in the prevalence rates of these autoantibodies studied could be due to different antigenic tissue substrates used and subject selection criteria.

Knowing that the normal population have detectable autoantibodies, the interpretation of laboratory results would be made simpler with a cut-off value. Taking into consideration that approximately 5% of the population have ANA, our cut-off for ANA (mouse liver) is 1:20, with HEp-2 cells is 1:40 and AMA is 1:20. This will also avoid the problem of false-positive results. The significance of autoantibody presence in asymptomatic individuals is unknown. Can this be taken to be an indicator of a pre-autoimmune state? Long term follow-up studies have to be undertaken to answer this problem. A larger study is suggested to compare the frequency of autoantibodies in different sex and age groups. Needless to say, screening for other autoantibodies is also needed to establish their frequency in the normal population.

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REFERENCES

1. Davis JS. Antinuclear antibodies (ANA). In: Kelly WN, Harris ED, Ruddy S, Sledge CB, eds: Textbook of Rheumatology, Vol 1. Philadelphia: WB Saunders Co. 1981: 671-709.
2. Yadin O, Sarov B, Naggan L, Slor H, Shoenfeld Y. Natural autoantibodies in the serum of healthy women - a five-year follow-up. *Clin Exp Immunol* 1989; 75: 402-6.
3. Fritzler MJ, Pauls JD, Kinsella TD, Bowen TJ. Antinuclear, anticytoplasmic and anti-Sjogren's Syndrome antigen A (SSA/Ro) antibodies in female blood donors. *Clin Immunol Immunopathol* 1985; 36: 120-8.
4. Siti Rohana BO, Ahmad IB, Nasruddin BA. Prevalence of antinuclear antibodies in Malaysian subjects. In: Proceedings of the 3rd Medical Colloquium, Medical Faculty, UKM, Kuala Lumpur 1992; 324-7.
5. Forslind J, Heigh Z, Jonsson J, Scheynius A. The prevalence of antinuclear antibodies in healthy young persons and adults, comparing rat liver tissue sections with HEp2 cells as antigen substrate. *Clin Exp Rheumatol* 1994; 12(2):137-41.
6. Andersen P. Correlation of smooth-muscle and nuclear antibodies in normal subjects. *Clin Exp Immunol* 1977; 27: 74-7.
7. Triger DR, Charlton CAC, Ward AM. What does the antimitochondrial antibody mean? *Gut* 1982; 23: 814-8.
8. Parker MD, Kerby GP. Combined titre and fluorescent pattern of IgG ANA using cultured cell monolayers in evaluating connective-tissue diseases. *Ann Rheum Dis* 1974; 33: 465-72.
9. Hooper B, Whittingham S, Mathews JD, Mackay IR, Curnow DH. Autoimmunity in a rural community. *Clin Exp Immunol* 1972; 12: 79-101.
10. Mcmillan SA, Haire M. Smooth muscle antibody in patients with warts. *Clin Exp Immunol* 1975; 21: 339-44.
11. Shu S, Nisengard RJ, Hale WL, Beutner EH. Incidence and titers of antinuclear, antismooth muscle and other autoantibodies in blood donors. *J Lab Clin Med* 1977; 86: 259-65.
12. Whitehouse JMA, Holborow EJ. Smooth muscle antibody in malignant disease. *Brit Med J* 1971; iv: 511-3.