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Prevalence of Positive Antinuclear Antibodies in Healthy Children

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SUMMARY Antinuclear antibodies (ANA) frequently arise in the sera of children with connective tissue disease and is used in the diagnosis of these diseases. Therefore it is also important to know the prevalence of ANA in normal children. The main objective of the present study was to determine the prevalence of antinuclear antibody (ANA) in healthy children. Ninety-nine serum samples from a serum bank and 108 samples from patients who had attended elective surgery and whose blood had been withdrawn for other investigations, were tested for ANA by indirect immunofluorescence method using HEp-2 cells as substrate. Sera from 52 children with SLE were also tested during the same period. It was found that antinuclear antibodies were present in 32 (15%) of the 207 sera of healthy children at a dilution of 1:40 or higher. ANA were positive in 9% at a serum dilution of 1:40, in 3% at 1:80 and in 3% at 1:160. The patterns of immunofluorescence staining were as follows: homogeneous in 46.7%, speckled in 20%, and nucleolar in 10%. In SLE patients, ANA were positive in 91%; 13% at a serum dilution of 1:40, 7% at 1:80, 20% at 1:160, 15% at 1:320, 9% at 1:640, 20% at 1:1,280 and 9% at ≥ 1:2,560. It was concluded that the prevalence of positive ANA using the HEp-2 cells as substrate was 15% in healthy children at dilutions of 1:40 or higher. Using the cutoff serum dilution of 1:40, the sensitivity of this test was 91%, the specificity was 85%, the positive predictive value was 57% and the negative predictive value was 97%.

Antinuclear antibodies (ANA) frequently arise in the sera of children with connective tissue disease,[1] including systemic lupus erythematosus (SLE), juvenile rheumatoid arthritis and juvenile dermatomyositis. Their serum presence is included in the classification criteria for SLE of the American College of Rheumatology. However, ANA can also be found in the absence of autoimmune diseases including various types of infections,[2-3] drug therapies,[4] neoplasia, pregnancy[5] and also in normal persons, especially the elderlies.[6]

There are many reports on the prevalence of serum ANA in normal adults,[7] but only few studies on their presence in normal children. The objective of this study was to determine the prevalence of ANA in normal Thai children.

MATERIALS AND METHODS

The study was conducted at the Department of Pediatrics and the Division of Immunology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand between March, 2003 and March, 2004.

The study protocol was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University. The sera used in this study were ob-

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tained from 2 groups of healthy children. The first group consisted of one hundred and eight serum samples from children, aged 6 months to 15 years, who were scheduled to have elective surgery (adenotonsillectomy, herniorrhaphy or plastic surgery), and whose blood samples were collected for other investigations (complete blood count, hemoglobin typing, etc.). Two milliliters of whole blood were obtained from individual children for this study after written informed consent had been obtained from their parents or legal guardians. The second group contained ninety-nine serum samples stored at -30°C from healthy children, aged 1 to 15 years prior to their vaccination since 1999. They were used after approval from the Ethics Committee. Sera from pediatric SLE patients, aged 5-15 years and diagnosed by using the 1997 revised criteria for the classification of systemic lupus erythematosus were used for comparison with the normal group.

We excluded patients who had overt autoimmune disease, or conditions associated with abnormal ANA titers (infection, hepatitis and malignancy) or underwent treatment with certain drugs (procainamide, hydralazine, chlorpromazine, isoniazid, methyldopa, penicillamine, quinidine, sulfasalazine, beta-blockers, sulfonamides). Children who were younger than 6 months of age were also excluded to avoid passive maternal antibodies.

Autoantibody assays

ANA were determined by a commercially available indirect immunofluorescence test kit (ANAFAST™ kits, Diasarin, Stillwater, MN, USA) using HEp-2 cells as substrate. The test was performed according to the manufacturer's instructions. Detection of ANA at a dilution of 1:40 was considered a positive result. All positive samples were titrated by serial 2-fold dilutions.

RESULTS

The normal children group consisted of 207 children with a mean age of 7.4 years (range from 7 months to 15 years). The female: male ratio was 1.13:1. The age and sex distributions of the study populations are shown in Fig. 1. Thirty-two children (15 males and 17 females; 15%) had a positive ANA test at a dilution titer of 1:40 or greater (Fig. 2). ANA were positive in 20 (9%) at a serum dilution of 1:40, in 6 (3%) at 1:80 and in 6 (3%) at 1:160. The patterns of ANA by indirect immunofluorescence were as follows: homogeneous 46.7%, speckled 20%, and nucleolar 10% (Table 1).

ANA were positive in 42 (91%) of the SLE patients; 6 (13%) at a serum dilution of 1:40, 3 (7%) at 1:80, 9 (20%) at 1:160, 7 (15%) at 1:320, 4 (9%) at 1:640, 9 (20%) at 1:1,280 and 4 (9%) at ≥ 1:2,560. Using the cutoff serum dilution of 1:40, the sensitivity of this test was 91%, the specificity was 85%, the positive predictive value was 57% and the negative predictive value was 97%.

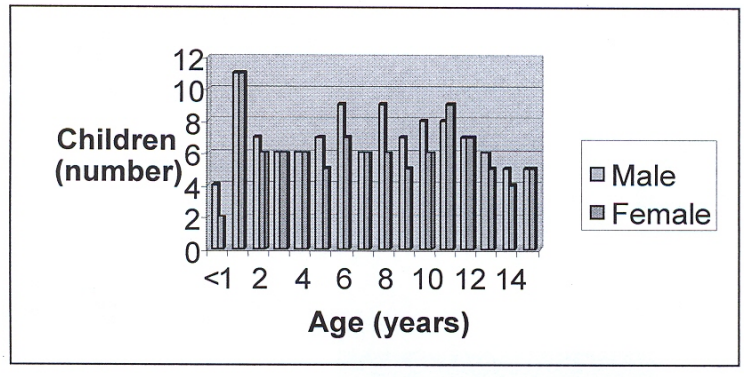

Fig. 1 Age and sex distributions of 207 normal children in this study.

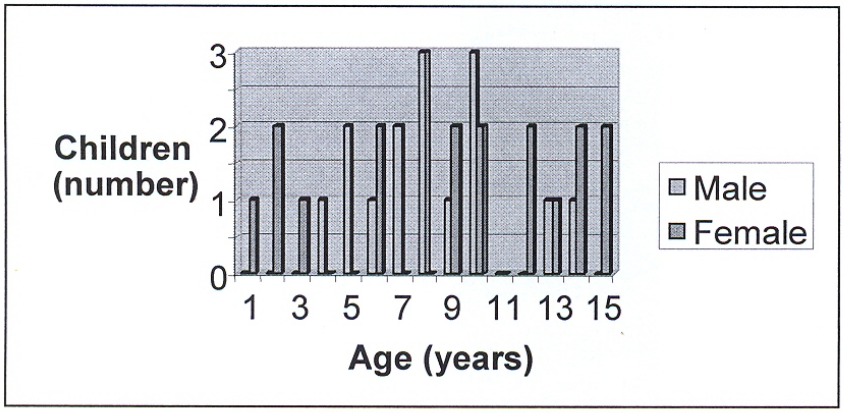

Fig. 2 Number of positive ANA in each age and sex.

Table 1 Immunofluorescent antinuclear antibody patterns found in 32 healthy children

Titer	First group	Second group	Pattern of ANA			
			Homogeneous	Speckled	Nucleolar	Combined
1:40	11	9	11	4	2	2 (homogenous, nucleolar), 1 (homogenous, speckled)
1:80	4	2	1	2	0	2 (homogenous, nucleolar), 1 (speckled, nucleolar)
1:160	2	4	3	1	1	1 (homogenous, speckled)
Total	17	15	15	7	3	7

First group: serum samples of children who had elective surgery.
Second group: serum samples taken from the serum bank.

DISCUSSION

The prevalence of ANA in healthy children in this study was 15%, which was higher than in most of the previous studies that used tissue substrates such as rat liver or mouse kidney as substrate (Table 2).[8-14] Our results are, however, comparable to a study that used HEp-2 cell as substrats.[13] The prevalence of ANA in this study was higher than in Youngchaiyud *et al.*[14] in 1981, who used leukocyte blood group O as substrate and included subjects from the age of 12 to 20 years. Arroyave *et al.*[10] demonstrated that the prevalence of ANA in normal children (undergoing plastic surgery) varied depending on the substrate used: 0.4% were ANA positive at a screening dilution of 1:20 using a mouse kidney substrate, compared to 0.8% ANA positive using HEp-2 cells at the same dilution.

The most common pattern of ANA found in healthy children in this study was homogeneous (46.7%), follow by speckled (20%) and nucleolar (10%). These findings were similar to data from Craig *et al.*[15] and Martini *et al.*[12]

Several studies[13,16,17] showed that children who had positive ANA results without autoimmune condition at their initial diagnosis would not develop an autoimmune diseases during a period of 38 to138 months of follow-up. Cabral *et al.*[13] and Deane *et al.*[16] concluded that it was not necessary to repeat ANA tests unless the patient develops other symp-

Table 2 Previous studies of the prevalence of ANA in healthy children

Study (year)	**Country**	**Age** (years)	**No.**	**Substrate**	**Screening dilution**	**No. positive ANA** (%)
Petty *et al*.[8] (1973)	Michigan, USA	NA	90	Rat liver	1:8	3 (3)
Goel *et al*.[9](1975)	Glasgow	0-15	112	Rat liver	1:16	0 (0)
Carlos *et al*.[10] (1988)	Chicago, USA	4/12-16	241	Mouse kidney	1:20	1 (0.4)
				HEp-2 cells	1:20	2 (0.8)
Baig & Shere[11](1989)	Saudi Arabia	1-19	184	NA	1:5	4 (2.2)
Martini *et al*.[12] (1989)	Italy	1/12-14	268	Rat liver & kidney	1:20	8 (3)
Cabral *et al*. [13] (1992)	Canada	6/12-18	93	HEp-2 cells	1:20	15 (16)
Youngchiyud *et al*.[14] (1981)	Thailand	12-20	74	Blood group O leukocytes	NA	2 (2.7)
This study (2004)	Thailand	7/12-15	207	HEp-2 cells	1:40	32 (15)

NA, no data available

toms or signs of autoimmune disease. Some studies showed that some of these children still had positive ANA with the same titers 8 to 24 months later[12,16] during a mean follow-up period of 61 months. However, Arbuckle *et al.*[18] found that 115 of 130 adult patients were ANA positive at a dilution of 1:120 or more, for a mean of 3.3 years before the diagnosis of SLE.

In this study, the test sensitivity was high (91%), but the specificity and positive predictive value were 85% and 57%, respectively. Even though there are many studies suggesting that children who have a positive ANA test without clinical symptoms of an autoimmune condition are at minimal risk to develop such a disease, the majority of patients with a positive ANA test have diagnosable autoimmune or rheumatic diseases that often respond to treatment, and ANA can be present years before clinical signs of SLE. We suggest that pediatric patients with a high titer of ANA should be regularly followed up and should have the test repeated if they develop a clinical manifestation suspicious of autoimmune disease, since they have a higher chance to develop the disease in the future.

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