FREQUENCY OF ANTINUCLEAR ANTIBODIES AND REHUMATOID FACTOR IN HEALTHY TURKISH CHILDREN*

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SUMMARY: Kasapçopur Ö, Özbakır F, Arısoy N, İngöl H, Yazıcı H, Özdoğan H. (Departments of Pediatrics and Rheumatology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, and Air Force Hospital, Etimesgut-Ankara, Turkey) Frequency of antinuclear antibodies and rheumatoid factor in healthy Turkish children. Turk J Pediatr 1999; 41: 67-71.

The frequency of antinuclear antibodies (ANA) and rheumatoid factor (RF) was investigated in 118 apparently healthy children (56 male, 62 female). The mean age was 9.8 ± 2.3 years. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence, using a Hep-2 cell substrate. Nephelometry was used to quantify RF in 116 children. Five serum samples (4%, 3M, 2F) were ANA-positive in low titers and all had a speckled pattern. None of the ANA-positive children had other extractable antinuclear antibodies. Rheumatoid factor (RF) was over 25 IU/ml in four children (3%, 3F, 1M). None of these was positive for both antibodies.

Our results suggest a similar frequency of ANA in healthy Turkish children even with a Hep-2 cell substrate, when compared to results of other reports. On the other hand, RF was more frequent than in other reported series. *Key words. antinuclear antibodies, rheumatoid factor, Turkish children.*

Positive antinuclear antibody (ANA) and rheumatoid factor (RF) test results are hallmarks of some autoimmune diseases like systemic lupus erythematosus, dermatomyositis, and rheumatoid arthritis[1-3]. These autoantibodies have been reported in healthy adults and children, also in relation to various infections and drugs[4-6].

The significance of a positive ANA test in an individual without an apparent autoimmune disorder is a topic of controversy[7, 8]. Using tissue culture cell substrates, usually Hep-2 cells, for ANA has increased sensitivity but decreased specificity, leaving us with a group of individuals with low titer, positive ANA tests[6, 9].

The effect of geographical distribution in the frequency of autoantibodies has also been discussed[4, 10]. We have shown previously that ANAs were less frequent in Turkish patients with juvenile chronic arthritis (JCA) when compared to series from the United States and the United Kingdom[11]. The present study was designed to determine the prevalence of ANA and RF positivity in a group of apparently healthy Turkish children.

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Material and Methods

This study was conducted in the Nurettin Ersin Primary School in Etimesgut, Ankara. The students attending this school included in this study were the children of military personnel, all of whom had a medium socioeconomic status. The school consisted of 12 classes and a total of 535 students (age range of 5 to 12 years). Ten children from each class were randomly selected. After the exclusion of two children with a recent history of infection, a total of 118 students (56 boys, 62 girls) were included in the study. The mean age was 9.8 ± 7.3 years (range 6-15 years).

The parents were asked to answer a standard questionnaire. No child with either a previous diagnosis of a disorder known to be associated with ANA positivity or with a recent history of infection within the last three months was included. All the children found eligible for the study underwent a physical examination by one of the authors (ÖK). Informed consent was obtained from the parents.

Sera taken from 118 children were stored at -20 °C until studied Antinuclear antibody (ANA) titer was determined by indirect immunofluorescence techniques on commercially prepared Hep 2 tissue slides (Kallestad Quantaflour). Specimens were diluted in phosphate-buffered saline (PBS), initially to 1:20. If positive, they were retested at subsequent dilutions. The diluted sera were incubated on the Hep-2 tissue slides for 10 minutes, at room temperature. They were then rinsed and washed in PBS for 10 minutes. Twenty-five 24 µl FITC conjugate were applied into the wells which were then incubated at room temperature for 20 minutes. This was followed by 10 minutes of further rinsing and washing with PBS containing Evans bule. The preparation was ready for examination after the application of buffered mounting media to the slides. They were examined at a magnification of 500x using a flourescent microscope (Leicca).

All sera positive for ANA in titers 1:20 and over were analyzed for antibodies to DNA, DNP, Ro, La, Sm, RNP and Scl-70. Rheumatoid factor (RF) was determined by nepholometry (Orion, Finland), as described elsewhere[12]. Results of 20 IU/ml and over were considered positive.

Children with positive ANA and/or RF were reexamined and tested for whole blood count, urinalysis, and erythrocyte sedimentation rate (ESR). Only the ANA-positive children underwent ophthalmological examination with biomicroscope.

Results

Antinuclear antibody (ANA) positivity at a dilution of 1:20 was detected in nine children (7.9%, 5M, 4F). Five of these were positive at a dilution of 1:40 (4.2%, 3M, 2F), and only one at 1:80 (0.8%). Nuclear flourescence was speckled in all. None of the nine children positive for ANA at a titer of 1:20 had antibodies directed against DNA, RNP, Sm, SS-A/Ro, SS-B/La or Scl-70 antigens.

Rheumatoid factor (RF) was positive in four children (3.3%, 1M, 3F). There were no children positive for both ANA and RF.

The initial physical examinations of the 118 children were normal. Thirteen children with a positive test result were reexamined and tested for whole blood count, urinalysis and ESR, all of which were found within normal limits. Children with ANA positivity were evaluated with biomicroscope by an ophthalmologist. All except one were normal. Examination of the girl who had a positive ANA titer of 1:80 revealed a sequela of chronic anterior uveitis. The family and the child denied any symptomatology related to this finding.

Discussion

We determined the frequency of ANA and RF in a total of 118 healthy children and found that four percent were positive for ANA at a titer of 1:40 and three percent for RF. There are a number of studies investigating the frequency of ANA in healthy children. The study population in the majority of these reports consists of children attending hospitals because of minor trauma or for simple surgical procedures[2, 4, 5, 9, 13]. Our study was a field survey and the study population consisted of primary school children with no recent history of infection.

Another important difference from the other reports was that all children were physically examined and the ones with ANA positivity underwent an ophthalmological examination[4, 5, 13]. With this approach we were able to detect a girl with a sequela of chronic anterior uveitis. She was also the child who had the highest ANA titer (1:80) in the study group. Her parents denied any joint manifestations since birth. She is being followed regularly by the ophthalmologist.

The positive ANA and RF results we detected in healthy school children were in general comparable with previous studies (Table I)[1-6, 10, 13-15]. The wide range of ANA positivity (0.8-18%) in healthy children might be the result of the different methods used and geographical factors[4, 5, 10].

Table I: Reported Series of Antinuclear Antibodies and Rheumatoid Factor Positivity in Healthy Children

Author	Year	ANA Researh Method	ANA Positivity (%) (n)	RF Research Method	RF Positivity (%) (n)
Petty et al.[1]	1973	Mouse liver	3 (3/90)	NI	NI
Goel et al.[2]	1975	IIF	0 (0/134)	Latex fixation	4 (5/134)
Osborn et al.[14]	1984	Hep-2 cell	8.5 (3/35)	NI	NI
Haynes et al.[15]	1986	Hep-2 cell	5.5 (1/18)	NI	NI
Arroyave et al.[9]	1988	Hep-2 cell Mouse kidney	0.8 (2/241) 0.4 (1/241)	NI	NI
Martini et al.[13]	1989	Rat liver	3 (8/268)	Latex fixation	05 (1/168)
Allen et al.[4]	1991	Hep-2 cell	18 (18/100)	NI	NI
Siamopolou-Mavridou et al.[10]	1991	Hep-2 cell	3 (2/66)	NI	NI
Forslid et al.[6]	1994	Hep-2 cell	1.3 (3/219)	NI	NI
Kanakoudi et al.[5]	1995	Hep-2 cell	2.2 (33/1500)	Nephelometry	1.2 (18/1500)
Present study	1997	Hep-2 cell	4.2 (5/118)	Nephelometry	3.3 (4/118)

NI: not investigated.

Allen et al.[4] reported an 18 percent ANA positivity in 100 healthy children at a titer of 1:40, which persisted at nine percent at a titer of 1:60. This finding has led to questions regarding the Hep-2 cell method. Contrary to Allen et al.'s report, in other studies utilizing the same method, the ANA positivity rate was similar to our findings[5,6,9].

In our children, 4.3 percent positivity rate at a titer of 1:40 declined to 0.8 percent at a titer of 1:80. A similar observation was reported by Forslid et al.[6]. Thus, attaining a positive result at a high or low titer is important for the evaluation of ANA positivity. Osborn et al.[14] detected a positivity rate of nine percent at a titer of 1:40; this rate decreased to null at a titer of 1:80. In the same study, the percentage of ANA positivity in children with juvenile rheumatoid arthritis (JRA) at a titer of 1:40 was 60 percent; the positivity rate declined to 31 percent at a titer of 1:80. Therefore, we suggest that a titer of 1:40 is useful in the evaluation of ANA positivity.

As Allen et al.[4] reported, in the postinfectious period, transient ANA positivity may be observed. However, none of the children in this study had a history of infection during the previous three months. In previous studies it has been reported that a higher ANA positivity rate was detected in girls, but in the present study there were no such differences[4,13]. It has also been reported that ANA positivity increases with age[13], but again, we did not observe such an increase.

In the Hep-2 cell method, ANA can be seen in four different forms: homogeneous, speckled, peripheral and nucleolar. The speckled view particularly shows the presence of other autoantibodies[6]. All five ANA-positive children had ANA positivity of the speckled type, but in none of them was a positive ENA detected. Although it has been reported that ANA positivity in an asymptomatic patient does not imply an uderlying autoimmune disorder, we detected an uveitis sequela in one of the ANA-positive patients[7,8].

Positive RF rates detected in this study were considerably higher than those detected in Italian and Greek children. This may reflect an increased sensitivity of the quantitative nephelometric method[5,13].

In conclusion, in Turkish children with JCA, RF and ANA positivity rates were lower than those in the British and North American series[11]. However, the results obtained in the healthy children were comparable to healthy populations from the above countries. We may conclude that the role of frequent infections and infestations in the positivity of ANA and RF is not as expected among Turkish children.

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