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ANTINUCLEAR ANTIBODIES AMONG EASTERN-POLISH RURAL INHABITANTS

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Abstract: Rural inhabitants are exposed to considerable amounts of pesticides from water, soil and air. There are indications that exposure to pesticides may trigger production of antinuclear antibodies (ANA). Therefore, the question arises about the prevalence of ANA in this group. In 90 eastern-Polish rural inhabitants (RI) and 50 urban blood donors (BD), the presence of ANA in serum was tested using EIA technique (Varelisa ReCombi ANA Profile). The frequency of ANA in the RI group was 2.5-fold higher than in the BD group ($p = 0.0175$). Among RI, at least one autoantibody was detected in 30% (95%CI: 20.5–39.5%). Most frequently, this was anti-dsDNA (12.2%; 95%CI: 5.5–19.0%), followed by SS-A/Ro (7.8%; 2.2–13.3%), RNP and Scl-70 (each 5.6%; 0.8–10.3%), Jo-1 (3.3%; 0.0–7.0%), Sm, SS-B/La, and CENP (each 2.2%; 0.0–5.3%). These figures are relatively high compared to studies of other random populations. In the BD group, at least one autoantibody was found in 12% (95%CI: 3.0–21.0%). Most frequently, this was anti-SS-A/Ro (8%; 95%CI: 0.5–15.5%), followed by dsDNA, RNP, and Scl-70 (each 2%; 0.0–5.9%). Neither Jo-1, Sm, SS-B/La, nor CENP-autoantibodies were found in this group. These figures place eastern-Polish blood donors in the middle of the range of prevalence observed among blood donors in other countries. The occurrence of antinuclear antibodies in eastern-Polish rural population is relatively high compared to both eastern-Polish blood donors and other random populations. A possible explanation for this is the long-term exposure to pesticides.

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INTRODUCTION

Antinuclear antibodies (ANA) are antibodies produced by the immune system that are directed against various structures located in the nuclei of the body's own cells. There is a convincing association between these antibodies and autoimmune diseases of the connective tissue, such as lupus erythematosus or scleroderma [19]. Production of ANA may be initiated by environmental chemicals [17], among these by pesticides [13]. As residents of agricultural areas are persistently exposed to pesticides from their environment, it appears reasonable to ask about the prevalence of ANA in this group.

The present study was aimed at comparing the frequency of antinuclear antibodies in two eastern-Polish populations: exposed to pesticides rural inhabitants, and blood donors from a big city.

MATERIALS AND METHODS

Study subjects. The study, carried out in 2000–2001, included 2 groups: The first group ("RI") comprised 90 residents of a rural community in Lublin Region (eastern Poland). This community is located in an exclusively agricultural area and there are no industrial emissions within the radius of 40 km. The RI group consisted of

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random persons who voluntarily attended a serological study of tick-borne encephalitis, for which Lublin Region is considered endemic [6, 7, 8]. Detection of ANA was carried out in the serum remaining from the mentioned study. The tests additionally were carried out in blood samples from 50 blood donors from the city of Lublin (group "BD"). Lublin (population approx. 370,000) is an administrative and educational centre in eastern Poland. The ecological status of the city is good; there are no emissions of environmental pollutants known to provoke autoimmune diseases.

Methods. Serum samples were tested for autoantibodies specific to the 8 following recombinant nuclear antigens: dsDNA, RNP, Sm, SS-A, SS-B, Scl-70, CENP, and Jo-1. The indirect, non-competitive enzyme immunoassay was used (Varelisa ReCombi ANA Profile, Pharmacia & Upjohn Diagnostics Freiburg, Germany) according to the manufacturer's instructions. The intensity of immune reaction was measured using ELISA reader ELX 800 (Bellco Biomedica Warsaw, Poland). The read-outs were considered positive if the absorbance ratio (sample read-out divided by cut-off value) was higher than 1.4. The difference in ANA frequency between both groups was tested for statistical significance using the double-sided t-test; frequencies of particular autoantibodies were expressed as percentages with 95-percent confidence intervals (95%CI) [12].

RESULTS

In the group RI (rural inhabitants) there were 25 men and 65 women, aged 18–82 (median 44) years. Blood donors (group BD) were all males aged 21–55 (median 35) years. At least one autoantibody was present in 30% of RI (95%CI: 20.5–39.5%), and in 12% of BD (95%CI: 3.0–21.0%). Thus, the overall frequency of ANA in RI was 2.5-fold higher as in BD, which was statistically significant ($p = 0.0175$). The autoantibody most frequently found in RI was anti-dsDNA (12.2%; 95%CI: 5.5–19.0%), whereas among BD - anti-SS-A/Ro (8%; 95%CI: 0.5–15.5%). Results for all ANA are shown in Table 1.

Table 1. Frequency of antinuclear antibodies among rural inhabitants and blood donors.

Antibody	Rural inhabitants (n = 90)			Blood donors (n = 50)		
	N	%	95% CI	N	%	95% CI
any antibody	27	30.0	20.5–39.5	6	12	3.0–21.0
dsDNA	11	12.2	5.5–19.0	1	2	0–5.9
SS-A/Ro	7	7.8	2.2–13.3	4	8	0.5–15.5
RNP	5	5.6	0.8–10.3	1	2	0.0–5.9
Scl-70	5	5.6	0.8–10.3	1	2	0.0–5.9
Jo-1	3	3.3	0.0–7.0	0	0	–
Sm	2	2.2	0.0–5.3	0	0	–
SS-B/La	2	2.2	0.0–5.3	0	0	–
CENP	2	2.2	0.0–5.3	0	0	–

N - number of cases with positive ANA, 95% CI - 95% confidence interval.

Table 2. Prevalence of antinuclear antibodies among blood donors according to various studies. Note that methods used, as well as criteria of positivity, vary from study to study.

Country	N	Method, positivity	% positive	Source
Oman	209	IIF, titre 1:20	2.9	[2]
Malaysia	93	IIF, titre 1:40	6.5	[3]
Sweden	255	IIF, titre 1:100	8.2	[24]
Poland	50	EIA	12	Present study
Belgium	485	IIF, titre 1:40	12.8	[10]
Canada	2500	IIF, titre 1:40	15.9	[11]
USA	183	IIF, titre 1:32	16.9	[9]

IIF - indirect immunofluorescence, EIA - enzyme immunoassay.

DISCUSSION

The frequency of ANA among eastern-Polish rural inhabitants was 2.5-fold higher than among blood donors. This difference should be treated with some caution as it may result in part from age and gender distribution: compared to BD group, the mean age in RI was higher (44 *vs.* 35 years) and there were more women (72% *vs.* 0%). Both these features positively influence the frequency of ANA [1, 25]. On the other hand, when comparing our results to other studies, it occurs that there must be also another factor responsible for the increased prevalence of ANA in rural inhabitants. While the results obtained in our blood donors place them just in the middle among similar populations, the prevalence in our rural population belongs to the highest in its class. Table 2 shows a review of studies previously carried out in blood donors: The frequency of ANA ranged from 2.9–16.9% (median 10.5%). Our results (12%) place eastern-Polish blood donors in the middle of the range (56th percentile). In contrast, the prevalence of ANA among eastern-Polish rural inhabitants (30%) exceeds figures from most other studies of random populations. Njemini *et al.* [15] reviewed such studies, published 1977–2002. Prevalence rates reported there ranged from 5–33% with the median of 11.2%. Our RI group with the prevalence of 30% would be placed over the 93rd percentile. Therefore, it appears that while the frequency of ANA in eastern-Polish urban blood donors came up to expectations, it was surprisingly high among eastern-Polish rural inhabitants.

Pesticides have long been an object of intensive biomedical research with respect to their toxic and allergising potential [14, 16, 32]. Recently, there has been increasing evidence that they are also capable of provoking autoimmune reactions [13], which may be related to the estrogenic activity of many pesticides [1]. In a population of Canadian farmers, Rosenberg *et al.* found a significant association between the presence of antinuclear antibodies and lifetime exposure to carbamate, organochlorine and pyrethroid insecticides, as well as to phenoxyacetic acid herbicides [18]. It has been shown

recently that up to 30% of sources of drinking water in rural areas of Poland are contaminated with pesticides; in most cases the concentration exceeds limits recommended by the European Union [5]. High contamination of air and the soil surface has also been observed as a result of pesticide spraying in orchards [4]. This shows that the rural environment in Poland is contaminated by considerable amounts of pesticides. From the humans' side, an indirect yet convincing indicator of the exposure may be found in Polish statistics of farmers' occupational diseases: Allergy to pesticides was diagnosed in 18% of all cases of occupational skin disease compensated from 1991–1999 [20]. For the development of allergy, a prolonged, repeated exposure to pesticides is required. Depending on external circumstances (exposure route, mode of exposure, cumulative dosis) and individual susceptibility, this prolonged exposure may also lead to autoimmune reactivity [13]. In combining the above, our results suggest that pesticide exposure of rural inhabitants may be responsible for increased production of ANA – autoantibodies that are widely accepted as markers of autoimmune diseases.

CONCLUSION

The occurrence of antinuclear autoantibodies is relatively high among eastern-Polish rural population, which probably results from long-term exposure to pesticides.

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