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ANTINUCLEAR ANTIBODIES IN BULGARIAN PATIENTS WITH CHRONIC INFECTIONS

Marin Zagorov, Marian Draganov, Stanimir Fransazov, Monica Trayancheva, Victoria Sarafian, Marianna Murdjeva*

Marin Zagorov - Department of Microbiology and Immunology, Medical University, Plovdiv, 4000 Plovdiv, 15A Vassil Aprilov Str.
Marian Draganov - Laboratory of Cell Biology, Faculty of Biology, University "Paisii Hilendarski" 4000 Plovdiv, 24 Tzar Assen Str.
Stanimir Fransazov - Faculty of Biology, University "Paisii Hilendarski" 4000 Plovdiv, 24 Tzar Assen Str.
Monica Trayancheva - Laboratory of Virology, Center of Disease Control, 4000 Plovdiv, 1 Perushtitza Str.
Victoria Sarafian - Department of Medical Biology, Medical University, 4000 Plovdiv, 15A Vassil Aprilov Str.
*** Marianna Murdjeva - Department of Microbiology and Immunology, Medical University, Plovdiv, 4000 Plovdiv, Vassil Aprilov 15A Str., E-mail: mmurdjeva@yahoo.com***
****Corresponding author***

ABSTRACT. The antinuclear antibodies are markers of autoimmune diseases, but they also appear in healthy individuals and in non-autoimmune diseases. The aim of this study was to evaluate the frequency of IgG antinuclear antibodies in patients with chronic infections, comparing different methods: 1. indirect immunofluorescent assay on rat liver tissue sections, HEp-2 cell line and on the serum-free McCoy-Plovdiv cell line; 2. enzyme-linked immunosorbent assay. Sera from 93 patients with chronic pneumonia and chronic viral hepatitis were screened for antinuclear antibodies through indirect immunofluorescent assay at 1/40 serum dilution on McCoy-Plovdiv and HEp-2 substrates. The positive ones were evaluated for antinuclear antibodies at higher titer to negative result. Forty eight of them were also evaluated through indirect immunofluorescent assay on rat liver and through enzyme-linked immunosorbent assay. At a titer 1/40 13% of the sera were positive for antinuclear antibodies on McCoy-Plovdiv, 6% - on HEp-2, 2% - on rat liver. At higher serum dilutions the number of positive sera decreased. There were no positive sera on enzyme-linked immunosorbent assay. The difference between the diagnostic

specificity of indirect immunofluorescent assay on cell substrates and indirect immunofluorescence on rat liver or enzyme-linked immunosorbent assay was significant as well as the difference in the frequency between antinuclear antibodies-positive men and women with chronic infections. There was no significant difference in the frequency between patients positive for antinuclear antibodies with chronic pneumonia and chronic hepatitis.

KEYWORDS. Antinuclear Antibodies, Indirect Immunofluorescent Assay, McCoy-Plovdiv cell line, Enzyme linked immunosorbent assay, Chronic infections.

INTRODUCTION

The antinuclear antibodies (ANA), particularly of IgG class, are markers of connective tissue diseases, but there are reports, that ANA (mostly of IgM class) are frequently detected among healthy individuals and in patients with non-autoimmune diseases (1,2). The aim of the study was to evaluate the frequency of IgG-ANA among patients with chronic infections with the standard detecting methods - indirect immunofluorescent assay (IIFA) on rat liver or HEp-2 cell line substrate and enzyme linked immunosorbent assay (ELISA), to estimate the specificity of these methods, and to compare it with the respective values of the IIFA test on McCoy-Plovdiv substrate - a novel serum- free cell line (3).

MATERIAL AND METHODS

Sera from 93 patients with chronic infections were used: 43 women and 50 men. Patients, included in the study were within the age range 3 months - 82 years. Fifty one of them were with chronic viral hepatitis and 42 with pneumonia. They were evaluated simultaneously for ANA through IIFA using McCoy-Plovdiv and HEp-2 cell substrates, and 48 of them were also evaluated through IIFA on rat liver and through ELISA. The substrates used for IIFA were as follows: McCoy-Plovdiv cells, grown on glass slides (in the laboratory) and acetone fixed as described (4, 5), HEp-2 cell slides (Binding Site, UK, cat. No FS 001.2); HEp-2000 cell slides (Immunoconcepts, USA, cat. No 2007-Ro); rat liver tissue cryostat sections, fixed on glass slides (in the laboratory). The FITC-conjugated goat anti-human IgG antisera were from: BulBio, Bulgaria (cat. No 444013), diluted 1/100 in phosphate buffered saline solution for antinuclear antibodies (PBS-ANA), and Immunoconcepts, USA (cat. No 2009-Ro), ready to use, for HEp-2000 slides. The IIFA-ANA was performed on McCoy-Plovdiv according to our protocol (5), while on rat liver, on HEp-2 and Hep-2000 as recommended by the producers. The initial dilution for IIFA was 1/40 in PBS-ANA. The positive sera were evaluated further at dilutions 1/80, 1/160, 1/320, 1/640 to a negative result. ELISA was performed on "ANA Screen Enzyme Immunoassay Test Kit", ClinPro International Co. LLC, USA (cat. No AD-702), with dilution, protocol, and cutoff value ($\geq$1,2) as recommended by the producer.

The following values were calculated: 1) OA (Overall agreement) - the proportion (in %) between the number of the matching results and the total number of the evaluated for ANA with two methods and 2) Diagnostic specificity - the proportion (in %)

between the number of the negative results and the total number of the evaluated for ANA patients with chronic infections by a given method. The statistical comparison between the diagnostic specificity of the methods was accomplished through descriptive statistics. The difference between the diagnostic specificity of the methods was assumed as statistically significant at $p \leq 0,05$.

RESULTS

The highest frequency of positive ANA results at titer 1/40 was detected on IIFA using McCoy-Plovdiv cell line as substrate (13%; 87% diagnostic specificity), followed by IIFA on HEp-2 cell line (6%; 94% specificity) (Table 6), although there is no statistical significance between both frequencies or specificities ($p>0.05$). The frequency of ANA positive IIFA on rat liver (4%; 96% specificity) was significantly different ($p<0.05$) from the results on McCoy-Plovdiv (Table 6). No positive sera were found on ELISA (100% specificity, significantly different from IIFA on McCoy-Plovdiv and HEp-2 at titer 1/40-1/160) (Table 6). The frequency of ANA, detected by IIFA on all substrates (McCoy-Plovdiv, HEp-2, rat liver) at titer 1/40 was highest (13%, 6%, 4%), followed by 1/80 (8%, 5%, 2%), 1/160 (5%, 4%, 2%), 1/320 (2%, 2%, 0%) and 1/640 (2%, 2%, 0%), i. e. the specificity is the lowest at 1/40, followed by 1/80, etc. (Table 6). The best OA (94%) was between IIFA on McCoy-Plovdiv and HEp-2 substrates (Table 1). The OA between IIFA using different substrates, or IIFA and ELISA increases with the increase of serum dilution (Tables 1-5).

DISCUSSION

The frequency of the positive ANA tests among patients with chronic infections, detected in other studies, varies between 3 and 25% (Table 9). It depends on the substrate and the studied population. Our data for positive IIFA on rat liver are comparable with the results of Kulthanan et al. (6) The difference between the results of Stoeger et al. (7) and ours may be due to the different populations, included in the study. Our results show that patients with chronic infections are frequently IIFA ANA-positive at dilutions <1/160, but rarely positive at dilutions ≥1/160. The probability that ANA (+) sera at dilutions <1/160 to be positive due to chronic infections, but not due to autoimmune disease, is higher than for ANA (+) sera at dilutions ≥1/160.

In our study we found that the positive results among female individuals are significantly more frequent than among male ones (Table 7). This corresponds with studies on healthy individuals (8). On the other hand, Muratori et al. (9) found that there is no correlation between non-organ specific autoantibodies (including ANA) positivity and age, sex and disease activity in chronic HCV patients.

Our data also show that the type of the chronic infection (e.g. viral hepatitis or pneumonia) has no significant influence on ANA-frequency (Table 8). It is supposed that not the type of the infection, but specific internal factors (probably HLA-genotype) of the organism are more important for the appearance of non-organ specific autoantibodies (9).

In our previous study we evaluated the frequency of ANA among healthy individuals (10). It is comparable to the frequency in patients with chronic infections by all methods. Similar results are obtained by Agarwal et al. (11).

CONCLUSIONS

The frequency of IgG ANA, detected in patients with chronic infections varies, when different detection methods (IIFA or ELISA) or different substrates for IIFA (tissue sections or cell layer) are used. The frequency depends also on the serum dilution. The serum-free McCoy-Plovdiv cell line, used as a cell substrate for ANA IIFA detection, has comparable diagnostic specificity with the standard substrate HEp-2, when serum samples of patients with chronic infections are evaluated.

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TABLES

Table 1. *OA between McCoy-Plovdiv and HEp-2 substrates at titer 1/40 - 1/640 (IIFA)*

Titer	Cell line			OA
	McCoy-Plovdiv	HEp-2		
		+	-	
1/40	+	6	6	94%
	-	0	81	
1/80	+	5	2	98%
	-	0	86	
1/160	+	4	1	99%
	-	0	88	
1/320	+	2	0	100%
	-	0	91	
1/640	+	2	0	100%
	-	0	91	

Table 2. *OA between McCoy-Plovdiv cell line and rat liver substrates at titer 1/40 - 1/640 (IIFA).*

Titer	Substrate			OA
	McCoy-Plovdiv	Rat liver		
		+	-	
1/40	+	1	9	79%
	-	1	37	
1/80	+	1	5	90%
	-	0	42	
1/160	+	1	3	94%
	-	0	44	
1/320	+	0	1	98%
	-	0	47	
1/640	+	0	1	98%
	-	0	47	

Table 3. *OA between IIFA using McCoy-Plovdiv at titer 1/40 - 1/640 and ELISA*

Method				OA
IIFA		ELISA		
		+	-	
1/40	+	0	10	79%
	-	0	38	
1/80	+	0	6	88%
	-	0	42	
1/160	+	0	4	92%
	-	0	44	
1/320	+	0	1	98%
	-	0	47	
1/640	+	0	1	98%
	-	0	47	

Table 4. *OA between HEp-2 cell line and rat liver substrates at titer 1/40 - 1/640 (IIFA).*

Titer	Substrate			OA
	HEp-2	Rat liver		
		+	-	
1/40	+	1	3	92%
	-	1	43	
1/80	+	1	3	94%
	-	0	44	
1/160	+	1	2	96%
	-	0	45	
1/320	+	0	1	98%
	-	0	47	
1/640	+	0	1	98%
	-	0	47	

Table 5. *OA between IIFA using HEp-2 at titer 1/40 - 1/640 and ELISA*

Method				OA
IIFA		ELISA		
		+	-	
1/40	+	0	4	92%
	-	0	44	
1/80	+	0	4	92%
	-	0	44	
1/160	+	0	3	94%
	-	0	45	
1/320	+	0	1	98%
	-	0	47	
1/640	+	0	1	98%
	-	0	47	

Table 6. *Frequency of ANA and comparison of diagnostic specificity between different methods.*
The values, marked with respective signs, have statistically significant differences (p<0.05) between each other (▲ - McCoy-Plovdiv / ELISA; ■ - McCoy-Plovdiv / Rat liver; ● - HEp-2 / ELISA).

Titer	Frequency %				Diagnostic specificity %			
	McCoy - Plovdiv	HEp-2	Rat liver	ELISA	McCoy-Plovdiv	HEp-2	Rat liver	ELISA
1/40	13 ■▲	6 ●	4 ■	0▲●	87 ■▲	94 ●	96 ■	100▲●
1/80	8 ▲	5 ●	2		92 ▲	95 ●	98	
1/160	5 ▲	4 ●	2		95 ▲	96 ●	98	
1/320	2	2	0		98	98	100	
1/640	2	2	0		98	98	100	

Table 7. *Frequency (%) of the positive IIFA results for ANA in every gender group by different detection methods (titer 1/40)*

gender	McCoy-Plovdiv		HEp-2		Rat liver	
	%	range	%	range	%	range
♀	23	1/40-1/640	14	1/40-1/640	4	1/160
♂	4	1/40-1/160	0	-	4	1/40

Table 8. *Frequency (%) of the positive results for IIFA-ANA in every disease group on different substrates*

Disease	McCoy-Plovdiv		HEp-2		Rat liver	
	%	range	%	range	%	range
Hepatitis	14	1/40 - 1/160	4	1/80 -1/160	4	1/40
Pneumonia	12	1/40 - 1/640	10	1/40 - 1/640	4	1/160

Table 9. *Frequency of IIFA positive results for ANA in patients with chronic infections, according to other authors*

Author	Diagnosis	Substrate	Positive ANA result
Kulthanan et al (6)	HIV	Rat liver	**3%** (titer1/40)
Stoeger et al (7)	chronic HCV	HEp-2	**25%** (titer1/40)
Muratori et al (9)	chronic HCV	Rat liver	**12,9%** (titer 1/40, polyvalent conjugate)
Agarwal et al. (10)	chronic HCV	Rat liver	**12%** (titer 1/10)