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Multicenter clinical and immunological study on Addison's disease in Poland**

Wieloośrodkowe kliniczne i immunologiczne badania u osób z chorobą Addisona w Polsce

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Keywords

Addison's disease, primary adrenal insufficiency, 21-OH antibodies, antiadrenal antibodies

Słowa kluczowe

choroba Addisona, pierwotna niedoczynność kory nadnerczy, przeciwciała przeciw 21-hydroksylazie, przeciwciała przeciwnadnerczowe

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Summary

Introduction. Primary adrenal insufficiency – Addison's disease (AD) is a rare disease, developing mainly due to autoimmune processes. Autoimmunity against 21-hydroxylase, an enzyme participating in steroidogenesis, is the most frequent pathogenic factor in AD.

Aim. Our study aimed at evaluating the incidence of 21-hydroxylase (21-OH) antibodies and presence of other immunological disorders in patients with primary adrenal insufficiency.

Material and methods. 212 patients (160 women) with hormonally documented Addison's disease, aged 20-84 years, observed for 1-52 years. Clinical examination, 21-hydroxylase and thyroid autoantibodies investigations. The results obtained in 2012 were compared with the results of adrenal autoantibodies measured in 2000 (then of 62 patients, adrenal antibodies were detected in 39 persons and 30 of them were investigated again in 2012).

Results. In 212 patients examined in 2012 antibodies to 21-OH were present in 114 cases (54%). Among 30 patients examined in 2000, 27 cases showed 21-OH positivity, whereas twelve years later antibodies were detected in only 13 cases. Presence of thyroid antibodies was the most frequent immunological finding, while hypothyroidism was the most frequent autoimmune disease.

Conclusions. 1. The incidence of positive results of 21-OH antibodies in 2012 = 54% appeared to be lower than in Scandinavian cohorts, probably due to long term observation in Poland. 2. A conversion from a positive immunological phenotype to a negative one during 12 years period of observation seems to confirm this suggestion.

Streszczenie

Wstęp. Pierwotna niedoczynność nadnerczy – choroba Addisona (AD), jest rzadką chorobą rozwijającą się na tle procesu autoimmunologicznego. Autoimmunizacja skierowana przeciwko 21-hydroksylazie, enzymowi uczestniczącemu w steroidogenezie, jest najczęstszym czynnikiem patogenetycznym w tej chorobie.

Cel pracy. Celem pracy było zbadanie częstości występowania przeciwciał przeciwko 21-hydroksylazie oraz częstości współistnienia innych chorób autoimmunologicznych u pacjentów z pierwotną niedoczynnością nadnerczy w Polsce.

Materiał i metody. Zrekrutowano 212 pacjentów (w tym 160 kobiet), z udokumentowaną badaniami hormonalnymi chorobą Addisona, w wieku 20-84 lat, obserwowanych przez 1-52 lat. Wykonano badanie kliniczne oraz oznaczenie przeciwciał przeciwko 21-hydroksylazie (21-OH) i przeciwciał tarczycowych (aTg i aTPO). Wyniki badań uzyskane w 2012 roku porównano z wynikami badania przeciwciał nadnerczowych wykonanych w 2000 roku (z 62 pacjentów 39 miało wówczas wynik pozytywny i 30 ponownie poddano badaniu w 2012 roku).

Wyniki. Z 212 pacjentów poddanych badaniu w 2012 roku obecność przeciwciał przeciwko 21-OH wykryto u 114 osób (54%). Wśród 30 chorych badanych pierwotnie

**This work was supported by EU project FP7 "Euradrenal" and CMKP grant 501-1-08-11/10-14. Authors express their thanks to Professor Olle Kämpe for measurement of adrenal antibodies and thanks to the listed contributing Polish endocrinologists for recruiting c. 30% of the total number of the studied patients.

w 2000 roku 27 miało te przeciwciała, natomiast ponowne badanie 12 lat później wykazało pozytywny wynik jedynie u 13 z nich. Wśród innych immunologicznych odchyleń najczęstsza była obecność przeciwciał tarczycowych, a najczęstszą towarzyszącą chorobą autoimmunologiczną – niedoczynność tarczycy.

Wnioski. 1. Częstość występowania przeciwciał przeciwko 21-hydroksylazie w grupie polskich pacjentów z chorobą Addisona (54%) jest niższa, aniżeli w materiale pacjentów skandynawskich, czego przypuszczalną przyczyną może być dłuższy okres obserwacji w naszej grupie chorych. 2. Konwersja wyniku dodatniego do ujemnego zaobserwowana u części pacjentów badanych ponownie po wielu latach może przemawiać za takim wytłumaczeniem.

INTRODUCTION

Primary adrenal insufficiency (Addison's disease – AD) is a rare disease, developing mainly due to autoimmune processes. Autoimmunity against 21-hydroxylase, an enzyme participating in steroidogenesis, is the most frequent pathogenic factor in AD. Clinical, immunological and genetic investigations have been conducted in the european project, Euradrenal, to study more deeply the pathogenesis of AD, its natural course and current possibilities of the therapy improvement. Clinical experience of the members of the Steering Committee resulted in an Expert Consensus Statement concerning diagnosis, treatment and follow-up of patients with AD (1).

At our department a tradition of addisonian patients' diagnosis, treatment and follow up, initiated in the sixties years of the 20th century, have been continued till now. Our observations were described in 1991 (2) and 2010 (3). In the first series of AD patients, including 180 cases, tuberculosis was diagnosed in 52 persons (29%). In the group of 138 patients, registered between 1990 and 2008, there were only 3 patients with tuberculous destruction of the adrenals.

Within Polish part of the Euradrenal program, combined groups of patients with autoimmune AD (AAD) observed in our department and 7 other endocrinological centers in Poland[1] have been studied.

AIM

The aim of this work was evaluation of the presence of 21-hydroxylase and thyroid (aTPO, aTg) autoantibodies as well as evaluation of the coincidence of other autoimmunological disorders.

The current results of 21-OH antibodies measurements were compared with adrenal autoantibodies (abs) determinations performed in 2000. Of 62 patients investigated initially, adrenal antibodies were positive in 39 cases. Thirty of them (five with associated primary ovarian failure – POF) were included in both studies. Such comparison enabled immunological follow-up of the autoimmune processes in Addison's disease.

MATERIAL AND METHODS

Our material included 212 patients, 160 women and 52 men, aged 20-84 years (at the time of the study), observed for 1-52 years. In this number, there were 148 patients from our department, and 64 patients from 7 other endocrinological centers. Diagnostic methods: clinical examination and hormonal determinations, which included: serum cortisol, DHEA-S, fT4, TSH, FSH, estradiol, testosterone, PTH (if necessary), plasma ACTH, urinary 17-hydroxycortisteroids (17-OHCS) or free cortisol and $^{1-24}$ACTH stimulation test.

Inclusion criteria: characteristic clinical features (4) and hormonal measurements – low serum cortisol at 9.00 hours, below 209 nmol/l (7.5 μg/dl) combined with high plasma ACTH concentration, over 13.2 pmol/l (60 pg/ml), decreased 17-OHCS urinary excretion, below 6.1 μmol/24 hours (2.2 mg/24 h), low peak serum cortisol level following $^{1-24}$ACTH (Synacthen) 0.25 mg *i.m.* or *i.v.* injection (1). Normal values: serum cortisol – 209 to 692 nmol/l (7.5-25 μg/dl), morning plasma ACTH – 3.3 to 13.2 pmol/l (15-60 pg/ml), urinary 17-OHCS – 6.1 to 19.3 μmol/24 h (2.2-7.0 mg/24 h).

Immunological studies: serum 21-hydroxylase (21-OH) autoantibodies (abs) determinations, thyroid autoantibodies (aTPO and aTg) measurements and a search for clinical data of other autoimmune disorders. The antibodies against 21-OH at first were determined by a method based on the *in vitro* transcribed and translated protein as described by Ekwall et al. (5). The upper normal limit of each antibody index was established as the mean value of negative controls plus three standard deviations and values above this cut-off indicated the presence of adrenal antibodies. Additionally, in 5 patients with POF, antibodies against 17-hydroxylase and against side-chain cleavage enzyme were measured (5).

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Analytical assays (if necessary): serum glucose, HbA1c levels, calcium and phosphorus, serum and urinary values, sodium and potassium levels.

RESULTS

The 21-OH antibody assays performed in 2012 detected positive responses in 114 out of 212 cases (54%). The time of observation ranged from 1 to 52 years; in 96 cases it exceeded 10 years. A comparison performed in the group of 27 patients with a significant titer of 21-OH autoantibodies detected in 2000 showed that in 2012 positive results remained only in 13 ones. The remaining 14 patients, which were positive in the first assessment became 21-OH antibodies (21-OH abs) negative in the last one. Such a negative conversion has been illustrated in the table 1. Among 5 patients with POF, the 17-OH abs and SCC antibodies were present in four, while negative in one patient (No 110) with premature menopause noted 48 years ago. Only in one patient with POF (No 115) positive 21-OH abs remained in 2012. In one patient (No 94), only significant anti-SCC abs were noted (without 21-OH abs). All patients presented in table 1 manifested other autoimmune disorders, the most frequently hypothyroidism, diabetes mellitus, vitiligo and POF.

Association of other autoimmune disorders was characteristic for majority of the addisonian patients under study. Thyroid abs, mainly aTPO, present in 180 patients (85%) have been the most frequent autoimmune finding. Table 2 shows the most frequent autoimmune diseases, with timing of their appearance. Thyroid diseases, hypothyroidism and thyrotoxicosis, were the most frequent disorders. In 25 patients thyrotoxicosis was followed by hypothyreosis, mainly in the cases treated by radioiodine. In single cases other autoimmune disorders were noted: rheumatoid arthritis, coeliac disease, Duhring's disease, chronic hepatitis, pericarditis. The analysis of timing of autoimmune disorders associated with AD did not show any significant differences, except of hypothyroidism, which followed AD in most cases. In 23 cases two autoimmune disorders accompanied AD, in 11 cases – three, while in two patients four other autoimmune disorders were associated with AD.

DISCUSSION

Most of the studies on the incidence of 21-OH antibodies in AD aims on diagnosis of its autoimmune form (6-8). Our investigations, including patients with long-time duration of AAD, aimed mainly on follow-up through the physiopathology of this disease and age-related modifications of the autoimmune reactivity. In our group there were 96 patients who have been observed at least for 10 years, which could influence the results of our studies.

Owing to the possibility to compare the same group of patients studied in 2000 and 2012 we were able to expose a conversion of a positive autoimmune phenotype of AAD in the first study to a negative one in the last series of immunological investigation. In 2000, in the group of 30 patients under study, 27 ones were positive for 21-OH abs while in 2012 only 13 of them remained positive. In the remaining group of 14 patients a negative immune phenotype appeared, interestingly – also in the women with high titer of 21-OH abs in 2000 (Nos 27, 68 and 132 in table 1). This observation suggests that an intensive autoimmune process could accelerate disappearance of autoimmune responses.

Table 1. Immunological data in patients with Addison's disease, studied in 2000 and 2012 (only patients with positive 21-OH or SCC antibodies in 2000).

Patients				Autoimmune disorders	Adrenal antibodies 2000			Adrenal antibodies 2012	Negative conversion
No#	Sex	Age	AD yrs	Types of disorders	21-OH	17-OH	SCC	21-OH	Yes or not
6	F	60	17	T-, DM, V	+			–	Yes
19	F	68	20	T-, DM	+			–	Yes
27	F	51	11	T+	+			–	Yes
39	F	77	11	T-, A	+			–	Yes
54	F	60	23	T-, POF	+	+	+	–	Yes
65	F	60	25	T-, V	+			–	Yes
68	F	41	10	T+, T-	+			–	Yes
74	F	85	37	T-	+			–	Yes
90	M	55	19	T-	+			–	Yes
94	F	59	26	T-, POF	–	+	+	–	–
110	F	76	11	T-, A, V, POF	+			–	Yes
115	F	54	23	T-, V, POF	+	+	+	+	Not
118	F	38	19	T+, T-, POF	+	+	+	–	Yes
125	F	55	15	T-, DM, V	+			–	Yes
127	F	40	13	DM, A	+			–	Yes
132	F	59	17	T-	+			–	Yes

– number on the list from 2012; T- – hyperthyroidism; DM – diabetes mellitus; A – anemia Addison-Biermeri; V – vitiligo; POF – primary ovarian failure

Table 2. Autoimmune disorders associated with Addison's disease in 212 patients under study.

Type of disorder	Number	%	Appearance		
			Before	Simult*	After
Hypothyroidism	106	50	26	25	55
Hyperthorodism	38	18	13	12	13
T1 Diabetes Mellitus	24	11	10	3	11
Vitiligo	23	11	10	5	8
Pernicious anemia	14	7	5	5	4
Premature menopause**	10	5	4	2	4
Alopecia	4	2	2	2	–
Moniliasis	3	1	3	–	–
Hypoparathyroidism	2	~1	–	2	–

*simultaneously
**in women only

It is well known from the literature that in diabetes mellitus most of the islet-cell cytoplasmic autoantibodies (ICA) disappear within initial 10 years; only in about 5% of cases the antibodies remain in the circulation (9-11). We suppose that in our patients in whom a negative conversion of immune phenotype was noted a similar mechanism took place. The destruction of adrenal tissue, due to autoimmunity, deprives the organism of the tissue antigens and the possibility to produce anti-adrenal autoantibodies. Diabetes mellitus, the most frequent endocrine disease of autoimmune origin could be a model of physiopathology of autoimmune processes.

An exception in our material, it was a 74-yrs old woman, observed for AAD for 52 years, who remained 21-OH abs positive. During that time she temporary suffered of hyperthyroidism. Thus immune reactivity is not age-related in all cases. Her sister's son (observed 18 years for AAD, and for hypothyroidism – 17 years) appeared to be 21-OH abs negative.

A selective production of anti-SCC abs only might explain a lack of 21-OH abs. In one our patient, with AAD and POF of 26 years duration (No 94 in table 1).

The relatively lower percentage of 21-OH abs presence in AD (54% in our last study), in comparison with the Scandinavian statistics (8) seems to be due to long duration of our observations. Including in this assessment 14 patients who were positive for 21-OH autoimmunity in 2000, and negative in 2012, the total number of 21-OH abs presence would increase up to 128/212 = 60%, which differs still evidently from the Scandinavian findings, probably due to evidently longer time of duration of AD in our series of patients.

Prevalence of thyroid autoimmunity in AAD has been observed by many authors, including our own experience (1-3, 6-8, 12). In our observations an increasing tendency to thyroid autoimmunity development in AAD was found (13). Diabetes mellitus and vitiligo took *ex aequo* the second place on our list of the incidence frequency (tab. 2), however only vitiligo preceeded AD even for 18 years. Pernicious anemia was the next on this list, usually associated with atrophic gastritis. Moniliasis, characteristic for APS1, was present in all three patients with this syndrome, while hypoparathyroidism only in two patients. A young man with AD, brother of a female patient with AD, hypoparathyroidism, T1DM and pernicious anemia, had only severe moniliasis. Mothers of the APS1 patients, included in the study, appeared to be 21-OH abs negative. In polyglandular insufficiency syndromes the associated disorders have been well tolerated by the patients except of T1DM, which worsened their self-estimation.

CONCLUSIONS

1. The incidence of positive results of 21-OH antibodies in 2012 = 54% appeared to be lower than in Scandinavian cohorts, probably due to long term observation in Poland.
2. A conversion from a positive immunological phenotype to a negative one during 12 years period of observation seems to confirm this suggestion.

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received/otrzymano: 30.10.2015
accepted/zaakceptowano: 23.11.2015