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Endocrine autoimmunity in patients with Latent Autoimmune Diabetes in Adults (LADA) — association with HLA genotype

Występowanie chorób autoimmunologicznych u pacjentów z późno ujawniającą się cukrzycą autoimmunologiczną u osób dorosłych — związek z genotypem HLA

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Abstract

Introduction: Latent autoimmune diabetes in adults (LADA) is a slow-developing form of autoimmune diabetes, characterised by the presence of type 1 diabetes-associated autoantibody and presentation at diagnosis similar to patients with type 2 diabetes. The aim of this study was to determine the prevalence of auto-antibodies related to endocrine autoimmune diseases in patients with LADA and to assess their association with HLA genotype.

Material and methods: We evaluated the presence of anti-thyroglobulin (ATG), anti-thyroid peroxidase (ATPO), anti-tissue transglutaminase IgA (ATTA), and anti 21 hydroxylase (A21H) in 70 patients with LADA, 69 with Type 2 diabetes, and in 50 healthy controls HLA genotype was assed in subpopulation of sluclied subjects.

Results: The presence of ATPO (28.6 *vs.* 10%); ATG (28.6 *vs.* 14%) was higher in patients with LADA in comparison to healthy controls and ATPO in comparison to patients with type 2 diabetes (38.6 *vs.* 17.4 %). In patients with LADA the presence of autoimmune thyroid autoantibodies was associated with newly diagnosed subclinical hypothyroidism; almost 7% of patients presented with high TSH. The presence of A21H (2.86 *vs.* 5.8 *vs.* 6.1%) and ATTA (2.86 *vs.* 4.3 *vs.* 6.0%) was not different between groups. Patients with high TSH level were positive for DQA1*0301 and DRB1*04 HLA genotype: DQB1*0201 and DQB1*02 were higher in patients positive for ATTA.

Conclusions: Patients with LADA have higher prevalence of thyroid autoimmune diseases.
In patients with LADA similarly to type 1 genotype DQA1*0301 seems to CONFER susceptibility to thyroid autoimmunity, and DQB1*0201 to celiac disease. **(Endokrynol Pol 2016; 67 (2): 197–201)**

Key words: *LADA; autoimmune thyroid; TSH; HLA*

Streszczenie

Wstęp: Późno ujawniająca się cukrzyca autoimmunologiczną u osób dorosłych cechuje się obecnością przeciwciał skierowanych przeciwko antygenom wysp trzustkowych u pacjentów z kliniczną prezentacją cukrzycy typu 2. Celem pracy była ocena występowania chorób autoimmunologicznych u pacjentów z cukrzycą LADA oraz ocena związku z genotypem HLA.

Materiał i metody: W obecnej pracy oceniono występowanie przeciwciał przeciwko-tyreoglobulinie (ATG), przeciwko peroksydazie tarczycowej (ATPO), przeciwko transglutaminazie tkankowej IgA (ATTA) oraz przeciwko 21 hydroksylazie (A21H) u 70 pacjentów z cukrzycą LADA, 69 z cukrzycą typu 2 oraz 50 osób z grupy kontrolnej oraz genotyp HLA.

Wyniki: Częstość występowania przeciwciał ATPO (28,6 *vs.* 10%), ATG (28,6 *vs.* 14%) była wyższa w grupie pacjentów z cukrzycą typu LADA w porównaniu do grupy kontrolnej oraz ATPO (38,6 *vs.* 17,4%) w porównaniu z pacjentami z cukrzycą typu 2. W grupie pacjentów z cukrzycą typu LADA istotnie cześciej stwierdzono obecność przeciwciał ATPO oraz ATG, którym towarzyszyly nowowykryte zaburzenia kliniczne funkcji tarczycy. Częstość występowania przeciwciał przeciwko 21 OH hydroksylazie (2,86 *vs.* 5,8 *vs.* 6,1%) oraz przeciwko transglutaminazie w klasie IgA (2,86 *vs.* 4,3 *vs.* 6,0%) nie różniła się pomiędzy badanymi grupami. Genotyp HLA DQA1*0301 występował częściej u pacjentów z autoimmunologiczną niedoczynnością tarczycy, nastomiast DQB1*0201 u pacjentów z przeciwciałami ATTA będącymi markerem choroby trzewnej.

Wnioski: U pacjentów z cukrzycą LADA częściej występują przeciwciała skierowane przeciwko antygenom tarczycy współistniejące z sublinicznią niedoczynością tarczycy. **(Endokrynol Pol 2016; 67 (2): 197–201)**

Słowa kluczowe: *cukrzyca LADA; choroby autoimmunologiczne tarczycy; TSH; HLA*

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Introduction

Latent autoimmune diabetes in adults (LADA) is a slowly progressing form of autoimmune diabetes, characterised by the presence of type 1 diabetes-associated autoantibody and presentation at diagnosis without acute clinical symptoms of insulin deficiency [1–3].

Similarly to Type 1 diabetes, patients with LADA have also a higher risk for endocrine autoimmune diseases. Patients with LADA have higher risk for thyroid and adrenal autoimmunity [4–7] and for celiac disease [6, 8, 9]. The presence of organ-specific autoantibodies is also higher in people with LADA and HLA-DR3-DQ2 antigens [5].

The aim of this study was therefore to determine the presence of antibodies associated with endocrine autoimmunity other than type 1 diabetes and asses its association with HLA genotype in patients with LADA.

Material and methods

The study protocol was approved by the Ethics Committee of the Medical University of Białystok. Informed consent was obtained from all patients and controls to be included in the study.

Diabetes was diagnosed according to the World Health Organisation (WHO) 1999 criteria. *Terms and Classifications:* those for whom insulin treatment was started within three months from diagnosis were classified as having type 1 diabetes. Diagnosis of LADA was made according to criteria proposed by *Immunology Diabetes Society and Action LADA* [1]. Those who were aged ≥ 30 years at diagnosis, were autoantibody positive, and insulin independent for a minimum of the first three months from diagnosis were considered to have LADA. Participants who were GADA- (glutamic acid decarboxylase antibody), IAA- (Anti Insulin Antibody), or IA2 (Insulinoma-associated antibody)-negative and insulin independent were considered to have type 2 diabetes. The current study consists of 70 patients with LADA, 69 with type 2 diabetes, and 50 healthy controls. The majority of patients were newly diagnosed with short duration of diabetes and did not have any known endocrine autoimmune disorders diagnosed before enrolment.

Laboratory measurements

Laboratory measurements were performed as previously described (10). TSH: thyroid stimulatory hormone (0.47–4.5 microIU/mL), anti-thyroglobulin antibodies ATG (IU/L), and anti-thyroid peroxidase antibodies ATPO (IU/L) were measured by microparticle enzyme immunoassay (MEIA) [11]. Anti 21 hydroxylase antibodies (A21H) were measured with RIA and anti tissue transglutaminase IgA (ATTA) antibodies with ELISA (cutoff higher than 20 reference units per ml). Any ATG positive antibody higher than two standard deviations from the mean in the whole studied population were consider as positive (cutoff 237 IU/L), similarly for ATPO (cutoff 49.5 IU/L) and A21H (cutoff 0.74 IU/L).

HLA genotype was performed as previously described [12].

Statistical methods

Differences in antibody frequencies were tested by the χ^2 test. Numerical data were expressed as mean and standard deviation unless indicated otherwise. Statistical significance was assessed by the Wilcoxon-Mann-Whitney test. To calculate the probability of the presence of positive ATPO and ATG by sex the Cochran-Armitage Trend Test was used.

A probability value of less than 0.05 was considered statistically significant. Analyses were carried out with SAS software (version 9.3, SAS institute, Cary, North Carolina).

Results

Clinical characteristics

Patients with LADA when compared to GADA-, IAA-, and IA2-negative diabetes were more likely to be younger, younger at the time of diagnosis, and had lower BMI and fasting C peptide levels. Patients with LADA when compared to healthy controls were more likely to be female and had lower fasting C peptide. Characteristics of patients with LADA, patients with GADA-, IAA-, and IA2-negative diabetes and healthy controls are depicted in Table I.

The presence of endocrine auto-antibodies

Mean plasma concentration for ATG, ATPO, and A21H were higher in LADA than in GADA, IAA-, and IA2-negative diabetes. Mean ATPO and A21H were also higher in LADA than in healthy controls (Table II).

Presence of ATPO (28.6 *vs.* 10 %); ATG (28.6 *vs.* 14%) was higher in patients with LADA in comparison to healthy controls and ATPO in comparison to GADA, IAA-, and IA2-negative diabetes (38.6 *vs.* 17.4 %) (Table II). Moreover, there was positive trend for ATG to be present more frequently among men and ATPO among women with LADA compared to both other groups (Table II). In patients with LADA the presence of thyroid autoimmune auto-antibodies was associated with newly diagnosed subclinical hypothyroidism; over 7% of patients with LADA had high TSH levels. The presence of A21H (2.86 *vs.* 5.8 *vs.* 6.1%) and ATTA (2.86 *vs.* 4.3 *vs.* 6.0%) was not different between groups (Table II).

Table I. *Baseline clinical characteristics in patients with LADA, GADA-, IAA-, and IA2-negative diabetes and healthy controls. Mean ± SD*

Tabela I. *Charakterystyka kliniczna pacjentów z LADA, pacjentów z cukrzycą typu 2 bez obecności przeciwciał (GADA, IAA, IA2) oraz w grupie kontrolnej*

	LADA	GADA, IAA-, and IA2-negative diabetes	Controls	LADA *vs.* GADA, IAA-, and IA2-negative diabetes	LADA *vs.* Controls
Number	70	69	50		
Age (years)	44 ± 13	49 ± 10	40 ± 6.9	0.008	0.252
Age at diagnosis (years)	40 ± 12	48 ± 11.5		< 0.001	
BMI [kg/m^2]	25.9 ± 4.5	29.5 ± 4.9	25.7 ± 3.1	< 0.0001	0.503
Sex F/M	41/29	38/31	20/30	0.677	0.044
Fasting C peptide [ng/mL]	0.67 ± 0.85	1.08 ± 1.27	1.68 ± 0.95	0.005	0.0001

BMI — body mass index; LADA — latent autoimmune diabetes in adults; GADA — glutamic acid decarboxylase antibody; IAA — anti insulin antibody; IA2 — insulinoma-associated antibody.

Table II. *Presence of endocrine autoantibodies in patients with LADA, GADA-, IAA-, and IA2-negative diabetes and healthy controls*

Tabela II. *Obecność przeciwciał charakteryzujących autoimmunologiczne choroby endokrynologiczne u pacjentów z LADA, pacjentów z cukrzycą typu 2 bez obecności przeciwciał (GADA, IAA, IA2) oraz w grupie kontrolnej*

	LADA	GADA, IAA-, and IA2-negative diabetes	Controls	LADA *vs.* GADA, IAA-, and IA2-negative diabetes	LADA *vs.* Controls
Mean ± SD ATG IU/L	225 ± 163	212 ± 145	144 ± 115	0.001	0.712
Mean ± SD ATPO IU/L	127 ± 217	38 ± 57	39 ± 104	< .0001	< 0.001
Mean ± SD ATTA IU/mL	8.34 ± 42.9	5.21 ± 7.93	5.31 ± 12	0.610	0.135
Mean ± SD A21H IU/L	0.29 ± 0.17	0.35 ± 0.23	0.26 ± 0.24	0.049	0.075
TSH > 4.5 microIU/mL	5/70 (7.14%)	1/69 (1.45%)	1/50 (2%)	0.099	0.202
ATG positive	20/50 (28.6%)	19/50 (27.54%)	7/43 (14%)	0.892	0.059
Female	9/32(12.9%)	12/26 (17.4%)	5/15(10%)	0.655*	
Male	11/18 (15.7%)	7/24 (10.1%)	2/28 (4%)	0.004*	
ATPO positive	27/43 (38.6%)	12/57 (17.4%)	5/45 (10%)	0.005	< 0.001
Female	20/21 (28.57%)	7/31 (10.14%)	3/17 (6%)	0.002*	
Male	7/22 (10.00%)	5/26 (7.25%)	2/28 (4%)	0.074*	
ATTA positive	2/68 (2.86%)	3/66 (4.3%)	3/47 (6%)	0.637	0.396
A21H positive	2/68 (2.86%)	4/65 (5.8%)	3/46 (6.1%)	0.394	0.382

*the Cochran-Armitage Trend Test; LADA — latent autoimmune diabetes in adults; GADA — glutamic acid decarboxylase antibody; IAA — anti insulin antibody; IA2 — insulinoma-associated antibody; TSH — thyroid stimulatory hormone; ATG — anti-thyroglobulin antibody; ATPO — anti-thyroid peroxidase antibody; A21H — Anti 21 hydroxylase antibody; ATTA — anti tissue transglutaminase IgA

Autoimmune antibodies in relation to HLA genotype

As expected, DQB1*0201 and DQB1*02 genotypes were higher in patients with antibodies positive for celiac disease (ATTA positive). Patients positive for Addison disease (A21H positive) had lower DQB1*0301/4 and DQA1*0501. Patients with high TSH levels were positive for DQA1*0301 and DRB1*04 (Table III).

Discussion

Latent autoimmune diabetes in adults (LADA), the slowly progressive form of autoimmune diabetes, is not a clear-cut disease but includes a population of patients with clinical presentation similar to type 2 diabetes but with autoimmune markers typical for type 1 diabetes [10]. In patients with LADA a higher rate of thyroid autoimmunity was extensively described [4–7] but not with association to HLA genotype. In our study patients with LADA also presented with higher rates for autoimmune thyroid disease markers, especially ATPO. Our results confirm the observations by Kucera et al. [6] that LADA had higher rate for ATPO, but not ATG in comparison to patients with type 2 diabetes. In agreement with a previous study, we found a higher prevalence of TPO antibodies in female LADA patients

Table III. *The association between HLA genotype and markers for endocrine autoimmune disease*

Table III. *Ocena związku haplotypu HLA z przeciwciałami charakteryzującymi autoimmunologiczne choroby endokrynologiczne w badanej grupie*

	Positive for celiac disease: ATTA positive	Negative for celiac: ATTA negative	Positive *vs.* negative
DQB1*0201	4/5 (80%)	26/111 (23.42%)	0.0047
DQB1*02	4/5 (80%)	38/111 (34.23%)	0.0373
	Positive for Addison disease: A21H positive	**Negative for Addison disease: A21H negative**	
DQB1*0301/4	0/8 (0%)	37/107 (34.58%)	0.0434
DQA1*0501	1/8 (12.50%)	55/107 (51.40%)	0.0337
	High TSH	**TSH in the normal range**	
DQA1*0301	3/3 (100%)	33/113 (29.20%)	0.0089
DRB1*04	3/3 (100%)	34/113 (30%)	0.0103

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[4]. Interestingly, in our study ATG was present more often among male LADA patients.

Additionally, in our study autoimmune thyroid antibodies were associated with high TSH; this may suggest the presence of subclinical hypothyroidism. More recently, Jin et al. [13] reported that a high titre of GADA was a strong predictor for the development of thyroid autoimmunity in Chinese patients with type 1 diabetes mellitus and LADA. In our analysis patients with TSH above the reference range presented with DQA1*0301 and DRB1*04, confirming their importance in susceptibility to thyroid autoimmunity [14, 15]. It is interesting to speculate that, additionally, viral infections could be playing a role in the development of thyroid autoimmune diseases and type 1 diabetes [16].

It is very well-established that patients with type 1 diabetes are predisposed to the presence of other endocrine autoimmune diseases, including thyroid disease. In the Polish type 1 diabetes population the presence of ATPO and ATG was describe as being as high as 16% [17]. Currently the Polish Endocrinology Society recommends active screening for thyroid disease in children with type 1 diabetes [18].

In patients with type 1 diabetes and LADA autoimmune markers for celiac disease are also present [6, 8, 9]. The presence of celiac disease confirmed by biopsy was estimated for about 10% in patients with type 1 diabetes in the Polish population [19]. Our analysis, contrary to previous observations, did not show a higher rate of celiac disease in patients with LADA [6, 8] and was in accordance with observations by Sanchez et al. [20].

Our study additionally confirms an association of the HLA genotype with autoimmune diseases. As expected, DQB1*0201 and DQB1*02 genotypes were higher in patients with antibodies positive for celiac disease and DQA1*0301, and DRB1*04 in patients with high TSH level. Similarly to previous studies, genotype DQA1*0301 seems to suggest susceptibility to thyroid autoimmunity, and DQB1*201 to celiac disease [14].

Conclusions

Patients with LADA have a higher prevalence of thyroid autoimmune markers. Screening for autoimmune thyroid diseases in patients with LADA similarly to type 1 diabetes can be proposed.

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