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Antithyroid immune status in different phenotypes of autoimmune diabetes

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Autoimmune diabetes is a spectrum of heterogeneous diseases united by the presence of autoimmune damage to pancreatic β-cells [1]. This spectrum includes, in addition to the classical type 1 diabetes mellitus (T1DM), with rapid destruction of β-cells and absolute insulin deficiency, latent forms such as latent autoimmune diabetes in adults (LADA) and latent autoimmune diabetes of the youth (LADY) [2, 3]. These phenotypes have significant differences. Classical T1DM usually debuts in childhood or adolescence, is characterized by acute clinical manifestations, often with ketoacidosis, high titers of autoantibodies (anti-GAD, anti-IA-2, ZnT8) and rapid development of insulin requirements [4].

LADA occurs in adults (usually after 30 years), has a slower course, the presence of autoantibodies (mainly anti-GAD), and does not initially require insulin [5]. LADY occupies an *intermedia*te position, debuting at a young age (16—29 years), combining signs of autoimmunity (often high titers and multiplicity of antibodies) with relatively preserved β-cell function at the beginning, which allows for the postponement of insulin therapy [6, 7].

An important aspect of the diagnosis and classification of these phenotypes is the presence of concomitant autoimmune diseases, which is considered as an additional diagnostic criterion, especially for LADA and LADY [8]. The most frequent companion of autoimmune diabetes is autoimmune pathology of the thyroid gland, in particular autoimmune thyroiditis (AIT) [9]. The presence of antithyroid antibodies (anti-TPO, anti-Tg) and the development of hypothyroidism not only worsen metabolic control in diabetes, but also serve as a marker of the general autoimmune alertness of the organism [10].

However, most studies are devoted to classical T1DM, while the features of antithyroid immunity in patients with LADA and LADY remain poorly understood. Clarifying this aspect may be of key importance for understanding the pathogenesis of autoimmune diabetes heterogeneity and improving approaches to the diagnosis and management of comorbidities.

Objective to determine the features of antithyroid immunity in patients with different phenotypes of autoimmune diabetes (classical T1DM, LADA, LADY) and to establish its relationship with parameters of carbohydrate metabolism, β-cell function, vitamin D status and anthropometric indicators.

MATERIALS AND METHODS

Study design and characteristics of groups. A total of 106 patients with autoimmune diabetes were examined, who were divided into three groups: group 1—40 patients with classical T1DM; group 2—36 patients with LADA; group 3—30 patients with LADY.

Diagnosis of autoimmune diabetes phenotypes was carried out according to current consensus [2]. The

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diagnosis of LADY was established on the basis of a set of clinical and laboratory data: age of onset from 16 to 29 years, positivity of autoantibodies to β-cells (GAD65, IA-2, ZnT8), C-peptide level at the lower limit of normal or moderately reduced, which allowed maintaining glycemic control without immediate prescription of insulin therapy.

All patients underwent standard clinical and laboratory examination monitoring, which included determination of anthropometric indicators (height, weight, body mass index (BMI), waist circumference (WC)) with calculation of waist circumference to height ratio (WHtR), carbohydrate metabolism (fasting glycemia by glucose oxidase method, glycated hemoglobin (HbA1c) by high-performance liquid chromatography method), assessment of β-cell function (serum insulin, C-peptide by immunochemiluminescence method) with calculation of insulin resistance index HOMA-IR, enzyme-linked immunosorbent assay (ELISA) determination of autoantibody levels (anti-GAD, anti-IA-2), assessment of antithyroid immunity and thyroid-stimulating hormone (TSH) by immunochemiluminescence method), as well as determination of 25(OH)D level in serum to assess vitamin D status by immunochemiluminescence method.

Statistical data processing was performed using Statistica 13.3 (StatSoft Inc.) and Microsoft Excel 2016. Quantitative data that did not obey normal distribution (Shapiro-Wilk test) were presented as median (Me) and interquartile range [Q25–Q75]. For comparison of independent samples, the nonparametric Mann-Whitney U-test was used. Qualitative analysis was performed using Fisher's exact test. To assess correlations with non-normal distribution, the Spearman rank correlation coefficient (r) was used. The difference was considered statistically significant at $p < 0.05$.

The study was approved by the Bioethics Commission of the Bukovinian State Medical University (protocol No. 2 dated 17.10.2019). All patients provided informed written consent to participate in the study.

RESULTS

Clinical and metabolic characteristics of the groups. The baseline characteristics of the examined groups are presented in Table 1.

The age of manifestation of DM in patients with LADA was significantly higher than in classical T1DM and LADY ($p = 0.000$). The duration of the underlying disease in patients with T1DM was 2.7 times longer than in LADA and twice as long as in patients with LADY.

The BMI of the T1DM and LADY groups was in the normal body weight category, while in LADA it corresponded to overweight. WHtR was also significantly higher in the LADA group compared to T1DM and LADY ($p = 0.000$), indicating more pronounced abdominal obesity in this group. Fasting plasma glucose and

Table 1

Baseline characteristics of the examined groups of patients with autoimmune diabetes mellitus

Indicator	Classical T1DM (n = 40)	LADA (n = 36)	LADY (n = 30)
Age, years	36.00 [27.00; 42.50]	49.00 [46.00; 55.00]*	29.00 [25.00; 31.00]**#
Age at diabetes onset, years	19.00 [13.50; 27.00]	41.00 [37.00; 45.50]*	20.00 [19.00; 23.00]#
Diabetes duration, years	15.00 [8.00; 21.00]	6.00 [3.00; 10.50]*	8.00 [6.00; 11.00]*
Women	18 (45 %)	20 (55.6 %)	17 (56.7 %)
BMI, kg/m^2	21.88 [21.00; 24.97]	26.61 [24.99; 29.21]*	24.16 [22.86; 26.30]**##
Waist/hip ratio	0.45 [0.43; 0.51]	0.53 [0.49; 0.55]*	0.45 [0.40; 0.48]#
Fasting plasma glucose, mmol/L	9.56 [8.08; 10.37]	9.37 [8.44; 10.78]	9.97 [8.32; 10.32]
HbA1c, %	8.85 [8.62; 9.38]	8.63 [7.55; 9.17]	8.50 [7.20; 9.40]
C-peptide, ng/mL	0.10 [0.10; 0.15]	0.60 [0.39; 0.84]*	0.62 [0.29; 0.82]*
Insulin, μU/mL	–	9.48 [8.30; 10.60] (n = 16)	8.01 [3.72; 11.95] (n = 16)
HOMA-IR index	–	3.88 [3.37; 4.64] (n = 16)	3.11 [1.65; 5.17] (n = 16)
anti-GAD, U/mL	351.75 [323.65; 417.18]	171.50 [95.50; 214.20]*	270.00 [252.00; 282.00]*#
25(OH)D, ng/mL	19.06 [17.65; 20.48]	18.85 [16.21; 23.13]	19.08 [15.03; 26.30]

Note. The difference from the classical T1DM is statistically significant: * $p < 0.001$; ** $p < 0.01$.
The difference from the LADA is statistically significant to: # $p < 0.001$; ## $p < 0.01$.

HbA1c levels did not differ significantly between the groups. The content of C-peptide in patients with T1DM was 6 times lower than in LADA and LADY ($p = 0.000$). Anti-GAD titers were the highest in patients with classical T1DM. 25(OH)D level in patients of all groups corresponded to the deficiency category and did not differ significantly.

Antithyroid immunity status. The results of the study of antithyroid antibodies and thyroid function are given in the Table 2.

Positive antibodies to TPO were recorded most often in patients with LADY (36.7 %), least often in LADA (27.8 %). Positive anti-Tg were detected with a similar, but lower frequency. The combination of two types of antithyroid autoantibodies was recorded in 20.0 % of patients with LADY, 17.5 % with classical T1DM and 13.9 % of patients with LADA.

The TSH level in patients with classical T1DM was the lowest among the study groups — 32.8 % lower than the LADA group and 18.1 % lower than in LADY ($p = 0.03$). The percentage of patients with subclinical hypothyroidism was the highest in the LADA group (13.9 %), slightly lower in LADY (10.0 %) and the lowest in individuals with classical T1DM (7.5 %). Manifested hypothyroidism was recorded with a similar frequency in classical T1DM (5 %) and LADA (5.6 %), and least often in LADY (3.3 %).

Correlation analysis. To identify relationships between antithyroid immunity indicators and other parameters, a correlation analysis was conducted separately for each group.

In T1DM group direct correlations of medium strength were found between the level of TSH and the age of the patients ($r = 0.328$; $p < 0.05$), the age of manifestation of DM ($r = 0.342$; $p < 0.05$), BMI ($r = 0.416$; $p < 0.05$). A negative correlation of medium strength was found between the level of 25(OH)D and HbA1c ($r = –0.318$; $p < 0.05$).

In LADA patients direct correlations of medium strength were found between the titer of anti-TPO and the level of HbA1c ($r = 0.543$; $p < 0.05$). Negative correlations were found between the level of 25(OH)D and WHtR ($r = –0.352$; $p < 0.05$), anti-Tg titer ($r = –0.359$; $p < 0.05$), as well as HbA1c ($r = –0.318$; $p < 0.05$).

The most numerous and strong correlations were registered in the LADY group. Inverse correlations of medium and strong strength were found between the level of 25(OH)D and BMI ($r = –0.437$; $p < 0.05$), WHtR ($r = –0.421$; $p < 0.05$), fasting glycemia ($r = –0.436$; $p < 0.05$), HbA1c ($r = –0.487$; $p < 0.05$), HOMA-IR index ($r = –0.583$; $p < 0.05$). A direct correlation was also found between the level of TSH and the age of manifestation of DM ($r = 0.563$; $p < 0.05$) as well as WHtR ($r = 0.398$; $p < 0.05$).

DISCUSSION

The results of our study confirm the hypothesis of phenotypic specificity not only of the course of autoimmune diabetes, but also of concomitant autoimmune damage to the thyroid gland. The data obtained demonstrate that the state of antithyroid immunity significantly depends on the clinical variant of autoimmune diabetes, which is important for the diagnosis and management of this patients.

The highest frequency of anti-TPO, the marker of greatest clinical significance in AIT, was found in the LADY group (36.7 %). This fact may indicate a more intense general autoimmune process in this category of patients, which is also confirmed by our data on a higher frequency of multiple diabetes-associated

Table 2

Indicators of antithyroid immunity and thyroid function in patients with different types of autoimmune diabetes

Indicator	Classical T1DM (n = 40)	LADA (n = 36)	LADY (n = 30)	p
Anti-GAD positive	39 (97.5 %)	31 (86.1 %)	28 (93.3 %)	0.150
Anti-IA-2 positive	19 (47.5 %)	10 (27.8 %)	14 (46.7 %)	0.110
Anti-TPO positive	13 (32.5 %)	10 (27.8 %)	11 (36.7 %)	0.040
Anti-Tg positive	9 (22.5 %)	7 (19.4 %)	7 (23.3 %)	0.120
Anti-TPO + anti-Tg	7 (17.5 %)	5 (13.9 %)	6 (20.0 %)	0.020
TSH, mIU/L	1.76 [1.32; 2.82]	2.62 [1.88; 3.99]	2.15 [1.67; 3.39]	0.030
Subclinical hypothyroidism	3 (7.5 %)	5 (13.9 %)	3 (10 %)	0.250
Manifested hypothyroidism	2 (5 %)	2 (5.6 %)	1 (3.3 %)	0.850

Note. Anti-TPO — anti-thyroid peroxidase, anti-Tg — anti-thyroglobulin antibodies.

antibodies compared to LADA. This is consistent with the data of other authors, who indicate a more aggressive autoimmune component in LADY compared to LADA [6, 11]. Thus, the presence of antithyroid antibodies, especially in young patients with diabetes, may serve as an additional argument in favor of the diagnosis of LADY.

It is interesting that, despite the lowest frequency of anti-TPO detection in LADA (27.8 %), the highest frequency of subclinical hypothyroidism (13.9 %) was observed in this group. This may be explained by the peculiarities of the metabolic status of patients with LADA, in which, unlike T1DM and LADY, are more likely to have signs of metabolic syndrome, in particular overweight and abdominal obesity (confirmed by the highest BMI and WHtR). It is known that insulin resistance and dyslipidemia can contribute to the progression of subclinical hypothyroidism to the manifest form regardless of antibody titers [12—14]. Our data on higher BMI, WHtR and the presence of insulin resistance in the LADA group confirm this hypothesis. The identified correlations were also phenotype-specific. For example, in LADA, a strong positive correlation was found between the anti-TPO titer and the HbA1c level, which may indicate a relationship between the activity of the autoimmune process against the thyroid gland and the deterioration of glycemic control, possibly due to the effect on the general pro-inflammatory status [15—17]. At the same time, in the LADY group, the most significant were the strong negative correlations between the level of vitamin D and glycemia as well as insulin resistance, which emphasizes the potential role of vitamin D deficiency in modulating both metabolic disorders and, possibly, autoimmune aggressiveness in this age group [18].

Vitamin D deficiency, which was found in all groups, is a common predictor of the development of both autoimmune diabetes and autoimmune thyroid pathology [19, 20]. In patients with autoimmune diabetes, it is associated not only with worsening glycemic control, but also with increased anxiety and depression [21, 22]. However, the nature of its interaction with clinical parameters, as shown by our study, differs depending on the autoimmune diabetes phenotype, which requires further study.

Thus, the study proves the existence of significant phenotypic differences in the state of antithyroid immunity in autoimmune diabetes, which is manifested by the highest frequency of anti-TPO carriage in LADY, the highest frequency of subclinical hypothyroidism in LADA and the phenotype-specific nature correlations between markers of autoimmunity, metabolic control and vitamin D status, which necessitates a differentiated approach to screening and management of such patients.

CONCLUSIONS

The state of antithyroid immunity differs significantly depending on the phenotype of autoimmune diabetes, which confirms the heterogeneity of the autoimmune process in these conditions and emphasizes the importance of assessing antithyroid antibodies as an additional criterion in differentiating phenotypes.

Patients with LADY are characterized by the highest frequency of carriers of antibodies to TPO (36.7 %) and their combination with anti-Tg (20.0 %), which indicates a high level of general autoimmune alertness in this group. Patients with LADA are characterized by the highest frequency of subclinical hypothyroidism (13.9 %) with a relatively lower frequency of antibodies, which may be associated with the influence of metabolic factors (overweight, abdominal obesity, insulin resistance).

The relationship between antithyroid immunity indices, glycemic control, vitamin D status and metabolic parameters is phenotypically specific, which requires an individual approach to the management of such patients. Mandatory screening for thyroid pathology (determination of TSH, anti-TPO) is indicated for all patients with autoimmune diabetes, regardless of its phenotype (classical T1DM, LADA, LADY), and the WHtR may be useful for assessing metabolic risk, especially in LADA.

Prospects for further research. A prospective study of the dynamics of antithyroid immunity in patients with different autoimmune diabetes phenotypes is promising to assess the prognostic significance of the identified changes, as well as to study the impact of correcting vitamin D deficiency on the course of both autoimmune diseases.

Conflicts of interest: none.
The authors declare no financial interest in the preparation of this article. The study was conducted in the absence of external funding.

Consent to participate. *Written informed consent was obtained from the participants.*

Authorship contributions: *conception and design — N. Pashkovska; acquisition of data — I. Tsaryk, N. Stankova, I. Bilooka; analysis and interpretation of data — I. Tsaryk, N. Pashkovska; drafting the article — I. Tsaryk; critical revision of the article — N. Pashkovska, V. Pankiv.*

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ABSTRACT

Autoimmune diabetes is a heterogeneous disease that includes several phenotypes, such as classical type 1 diabetes mellitus (T1DM), latent autoimmune diabetes in adults (LADA) and latent autoimmune diabetes in the youth (LADY). The presence of concomitant autoimmune diseases, in particular thyroid pathologies, is one of the key criteria for their differentiation and diagnosis.

Objective — to determine the features of antithyroid immunity in patients with different phenotypes of autoimmune diabetes and its relationship with clinical and metabolic parameters.

Materials and methods. 106 patients with autoimmune diabetes were examined: 40 with classical T1DM, 36 with LADA and 30 with LADY. All patients were tested for antithyroid antibodies (anti-TPO, anti-Tg), thyroid-stimulating hormone (TSH), diabetes-associated antibodies (anti-GAD, anti-IA-2), C-peptide, HbA1c, vitamin D, and anthropometric parameters (BMI, waist-to-height ratio).

Results. The frequency of anti-TPO detection was highest in the LADY group (36.7 %), *intermedia*te in T1DM (32.5 %) and lowest in LADA (27.8 %). The coexistence of anti-TPO and anti-Tg antibodies was observed in 20.0 %, 17.5 % and 13.9 % of cases, respectively. The highest frequency of subclinical hypothyroidism was found in LADA (13.9 %). Several phenotype-specific correlations were identified, in particular, a strong negative correlation between vitamin D and HbA1c in LADY ($r = –0.487$; $p < 0.05$).

Conclusions. The status of antithyroid immunity differs significantly depending on the autoimmune diabetes phenotype. LADY is associated with the highest frequency of anti-TPO carriage, indicating a more intense general autoimmune process. In LADA, subclinical hypothyroidism is more frequent, possibly related to metabolic factors. Screening for thyroid pathology should be considered in all patients with autoimmune diabetes.

Keywords: autoimmune diabetes, type 1 diabetes mellitus, latent autoimmune diabetes in adults, latent autoimmune diabetes in the youth, antithyroid antibodies, hypothyroidism, vitamin D.

РЕЗЮМЕ

Стан антитиреоїдного імунітету залежно від фенотипу автоімунного діабету

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Автоімунний діабет (АД) є гетерогенним захворюванням, що охоплює кілька фенотипів, зокрема класичний цукровий діабет (ЦД) 1 типу, латентний АД дорослих (ЛАДД) і латентний АД молоді (ЛАДМ). Наявність супутніх автоімунних захворювань, зокрема патології щитоподібної залози, є одним із ключових критеріїв їхньої диференціації та діагностики.

Мета — вивчити особливості антитиреоїдного імунітету в пацієнтів із різними фенотипами АД та його взаємозв'язок із клініко-метаболічними параметрами.

Матеріали та методи. Обстежено 106 пацієнтів з АД: 40 із класичним ЦД 1 типу, 36 із ЛАДД та 30 із ЛАДМ. У всіх пацієнтів визначали рівні антитиреоїдних антитіл (anti-TPO, anti-Tg), тиреотропного гормона, діабет-асоційованих антитіл (anti-GAD, anti-IA-2), С-пептиду, HbA1c, вітаміну D, антропометричні показники (індекс маси тіла, співвідношення обводу талії та зросту).

Результати. Частота виявлення anti-TPO була найвищою в групі ЛАДМ (36,7 %), проміжною при ЦД 1 типу (32,5 %) та найнижчою при ЛАДД (27,8 %). Комбінацію anti-TPO й anti-Tg зареєстрували в 20,0, 17,5 та 13,9 % випадків відповідно. Найвища частота субклінічного гіпотиреозу виявлена при ЛАДД (13,9 %). Установлено численні фенотипово-специфічні кореляційні зв'язки, зокрема сильну негативну кореляцію між вітаміном D та HbA1c при ЛАДМ ($r = –0,487$; $p < 0,05$).

Висновки. Стан антитиреоїдного імунітету суттєво відрізняється залежно від фенотипу АД. ЛАДМ асоціюється з найвищою частотою носійства anti-TPO, що вказує на інтенсивніший загальний автоімунний процес. При ЛАДД частіший субклінічний гіпотиреоз, можливо, пов'язаний із метаболічними чинниками. Скринінг патології щитоподібної залози показаний усім пацієнтам з АД.

Ключові слова: автоімунний діабет, латентний автоімунний діабет дорослих, латентний автоімунний діабет молоді, антитиреоїдні антитіла, гіпотиреоз, вітамін D.

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