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An algorithm for preclinical diagnosis of type 1 diabetes as a basis for creating the Register of DAA-positive children and adolescents of Ukraine with predicted development of disease

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Abstract. Aim — The establishment of mechanisms for T1D development at early and late preclinical stages of disease formation in children and adolescents. **Material and methods.** At the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of NAMS of Ukraine» mentioned the Program «Immunity in the preclinical period of T1D development» was initiated, on the basis of which the Register of marker-positive children with predictable development of type 1 diabetes was created, which includes 612 children aged from 7 to 15 years with burdened heredity, in which the titer of diabetes-associated autobodies (DAA), cytokines, levels of basal and postprandial glycemia and secretion of C-peptide at preclinical and clinical stages of T1D development in children and adolescents based on the performed clinical and immunological study. **Results.** The new data have been obtained at the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of NAMS of Ukraine», which allowed to substantially supplement the existing ideas about the type 1 diabetes (T1D) pathogenesis. As a result of the performed study, a group of marker-positive children with burdened heredity and a predicted risk of developing the disease was formed. It was found that an increased titer of DAA was observed in 162 (35.45%) of 457 children with burdened heredity with no less than two times determination of DAA presence in them, mainly GADA and IA-2A, the clinical debut was manifested in 86 (53.08%) of them from 6 months to 16 years (27.4±4.3 months). The formula of combined occurrence and

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values of simultaneously increased DAA titers to islet autoantigens, namely IA-2A + GADA, was determined, which is a predictor of both the duration of preclinical stage of T1D development and the debut rate. Impaired cytokine production (increase of the level of proinflammatory cytokines IL-1α, IL-6 and TNFα, IL-8 and IL-16 while reducing the concentration of IL-4 in the PB) as key factors of the T1D pathogenesis, which determine the rate of T1D debut, and the aggressiveness of its course were also established. It was found that the early preclinical period of T1D development in DAA+ children was characterized by the presence of dysglycemia in the form of increased glycemia in 2 hour after the glucose tolerance test and a slight decrease in secretion of stimulated C-peptide; in addition, dysglycemia in the form of impaired fasting glycemia was added in DAA+ children in the late preclinical period, and a decrease in both basal and stimulated secretion of the C-peptide was determined, indicating that the potential of pancreatic beta cells was depleted. **Conclusions.** Data on the establishment of mechanisms for T1D development at early and late preclinical stages of disease formation. the identified significant changes in innate and acquired immunity, immunological, metabolic and hormonal parameters are important both at the early and later preclinical stages of T1D development, indicating that the period of chronologically increasing consecutive breakdowns in different links of immunity significantly ahead time of the clinical debut of T1D, which can be used as predictors of the disease development. The Register of marker-positive children with predictable development of type 1 diabetes was created.

Keywords: type 1 diabetes, children and adolescents, diabetes-associated autoantibodies (DAA), glutamic acid decarboxylase autoantibodies (GADA), tyrosine phosphatase protein (IA-2A), cytokines, basal and postprandial glycemia, basal and stimulated C-peptide.

Introduction

Type 1 diabetes (T1D) is classified as the medical and social problems, which determine the priorities for the development of modern medicine, due to the constantly progressing an increase in the incidence of this pathology, the magnitude of its prevalence, early disability and high mortality of patients [1]. The priority actuality of this problem causes the resonance of clinical and immunological studies on the mechanisms of T1D evolution, and diagnosis at different stages of the disease formation and the development of new approaches to its prevention and treatment [2-9]. Studies of immunological markers as predictors of T1D development, which include the diabetes-associated autoantibody (DAA) to the islands of Langerhans (IL), and cytokines, as selective screening in clinical practice, are of primary importance [10-17]. The DAA determination has become an integral clinical tool for predicting the possible development of T1D in a still practically healthy subjects, a more in-depth study of latent immunological mechanisms in the latent preclinical period, which leads to T1D manifestation in humans and screening for selecting the patients to risk group long before of their disease development [18, 19]. The widespread introduction of DAA study into the practice of clinical endocrinology and the standardization of methods for their determination according to WHO protocols resulted in the creation of national multicenter long-term programs for studying the mechanisms of preclinical T1D development [20]. Until recently, the prospective studies on the identification of patients positive for DAA content at the latent stage of disease formation in children and adolescents with burdened heredity in relation to T1D, and the state of carbohydrate metabolism and insulin-producing function were not performed in Ukraine. The directions of our study vector are also due to the fact that the scientific literature data available to date mainly cover the analysis of similar indicators of immunity in children diagnosed with both newly and long-term diagnosed T1D, while an information on the state of immune mechanisms in preclinical latent period of this pathology development is extremely limited and contradictory. The actuality for studying the clinical and immunological aspects of T1D development and preclinical diagnosis in children and adolescents was caused by the above-mentioned data.

The purpose is to establish the clinical and immunological mechanisms of type 1 diabetes

pathogenesis at the early and late preclinical stages of its development, and to develop the new approaches to preclinical diagnosis of the disease in children and adolescents.

Material and methods

To assess the state of carbohydrate metabolism, the level of fasting venous plasma glucose was determined using the glucose oxidase method in an accredited laboratory. Capillary or venous plasma glucose levels were evaluated by the glucose oxidase method or the Supreme Petit glucometer with Hypoguard Supreme strips. If fasting glycemia was measured in whole blood, the corresponding plasma glucose concentration was calculated by the equation: $Gfp=Gfc\times1.11$ (mmol/L), where Gfp is the fasting plasma glucose concentration, Gfc is the fasting capillary glucose concentration. Fasting glucose limits in the capillary blood by this method were 3.6-5.5 mmol/L. To exclude or confirm the impaired carbohydrate tolerance, the oral glucose tolerance test (OGTT) was performed in all the examined subjects, according to the method, given in order № 582 of the Ministry of Health of Ukraine dated 15.12.2003. When assessing OGTT results, glucose tolerance was considered to be normal if its level in fasting plasma was less than 6.1 mmol/L and 2 hours after glucose loading — less than 7.8 mmol/L then the condition was classified as impaired glucose tolerance. If fasting glucose level did not exceed 6.1 mmol/L, and after 2 hours ranged from 7.8-11.1 mmol/L, the condition was classified as impaired glucose tolerance. The assessment of compensation degree for impaired carbohydrate metabolism in T1D development was performed in accordance with the recommendations [21]. The level of glycated hemoglobin (HbAlc) in the blood was examined to assess the compensation of carbohydrate metabolism in children and adolescents with T1D, and ascertaining the metabolic marker presence at preclinical stage of disease. HbAlc content was determined by the BTS-330 Semi-Auto biochemical analyzer using Glycated hemoglobin kit (Lachema, Czech Republic). The radioimmunological method to determine DAA: GADA, IA-2A, and IAA was used for detecting the presence of an autoimmune process in the pancreas. The number of concentrations in examined DAA in the blood was determined according to the manufacturer's instructions using special kits for detecting their content (Immunotech, Czech Republic and CIS Bio International, France) by the Beckman 5500B Gamma Counter (USA). The normal level of autoantibodies — GADA and IA-2A was less than 1 U/ml, and the level of autoantibodies — IAA was less than 5.5 U/ml. The concentration of different types of cytokines (IL-1α and β, IL-4, IL-6, IL-10, TNFα, IFNγ) and chemokines (IL-8 and IL-16) in PB was studied by enzyme-linked immuno sorbent assay (ELISA) using Stat Fax 3200 spectrophotometer (USA), and reagent kits from Diaclone (France).

Radioimmunoassay (RIA) method was used to determine the C-peptide content in the blood of sick and healthy children. According to the attached instructions, RIA kits were used for determining the serum and plasma hormone concentrations: IMMUNOTECH (Czech Repub-lic), radioactivity was calculated by the Beckman 5500B Gamma Counter (USA).

The calibration curves were automatically built. The results obtained were considered valid if the coefficient of variation did not exceed 10% within and between the series. Levels of C-peptide within 0.644-2.83 ng/ml were considered normal. Statistical analysis of the data was performed on the grounds of Microsoft Excel and SPSS11 application packages (SPSS Inc., USA) using parametric methods for statistical analysis. Under the condition of a normal distribution of data, they are presented both in the form of arithmetic mean values with a standard deviation as well as from arithmetic mean value (M±σ), and mean values and their standard error (M±m). Student's t-test was used to compare two groups according to quantitative normally distributed by characters. The differences were considered significant when confidence level was p<0.05. All the statistical calculations were performed at significance level of 95%, p=0.05. The critical level of significance for testing statistical hypotheses when comparing groups was assumed to be 0.05.

Clinical characteristics of the examined subjects. Within the State Program «Diabetes mellitus» there were examined 612 children and adolescents of both sexes aged from 7 to 15 years (mean age — 12.34±0.82 year). Of the 612 practically healthy children who were

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examined, the main group included 457 (74.67%) children and adolescents with normoglycemia who had the relatives with first-degree of T1D: parents, siblings. This group of children, according to the radioimmunoassay performed for the presence of simultaneously increased titers of DAA, such as: IAA, IA-2A and GADA was divided into two large subgroups. The DAA-positive group consisted of 162 (35.45%) children; the necessary inclusion criterion was the obligatory presence, by the double determination, of simultaneously increased titer of at least two types of DAA for Li antigens, predominantly GADA and IA-2A ones. The DAA-negative (DAA-) group consisted of 295 (74.32%) patients with a normal glycemic level and the absence of simultaneously increased titer of DAA during a double examination at the beginning of a prospective observation. Another group appeared as the logical chronological completion of the autoimmune process in the group of DAA-positive (DAA+) children — a group of children with T1D debut. It was made up of 86 (72.34%) children, who gradually changed their «normoglycemic» DAA-positive status of practically healthy children to the status of patients with clinical debut of T1D during 6 monthes — 15 years (average 27.4±4.3 month). Another large observation group consisted of 143 patients with T1D of different disease duration and degree of compensation. The control group included 155 practically healthy normoglycemic children (25.32%).

Results and discussion

The highest-frequency detection of all three types of DAA was diagnosed in the DAA+ children group: IAA — in 149 (92.56%), GADA — in 137 (84.61%), and IA-2A — in 23 (76.59%) children. IAA were identified in 73 (85.71%), GADA — in 67 (78.26%), and IA-2A — in 35 (70.58%) children of the group of DAA+ ones with clinical debut of disease. IAAs were detected in 53 (69.2%), GADA — in 65 (69.1%), and IA-2A — in 47 (50%) children from the group of patients with T1D debut who were not included in clinical-immunological examinations. There were found 33.3% of IAA, 46.6% of GADA, and 33.3% of IA-2A in the group consisting of 15 children with T1D duration from 1 to 5 years. In the group which included 14 children with

disease duration more than 5 years IAA were determined in 24,42%, GADA — in 35,71%, IA-2A — in 14,8% of children, and in the group of 10 children with disease duration more than 10 years IAA were detected in 12.07%, GADA — in 24.12%, IA-2A — in 7.13% of children. As one of the criteria for inclusion of children with hereditary burden of T1D into DAA+ group was the presence of combined double increase of at least two types of DAA, the mean values of the initial and final (pre-debut) levels of DAA titers — IAA, GADA and IA-2A were determined by us in children in the preclinical period of T1D development. It was shown that the highest titers of autoantibodies: IA-2A — 18.96±1.95 U/ml, GADA — 17.26±1.95 U/ml, IAA — 14.82±1.372 U/ml compared to similar values in the group of DAA-positive children at the early stage of T1D development: IA-2A — 6.83±0.88 U/ml (p<0.001), GADA — 12.46±1.36 U/ml (p<0.001), IAA — 9.04±0.77 U/ml (p<0.001), and in children with clinical debut of T1D: IA-2A — 12.65±1.40 U/ml (p<0.001), GADA — 13.69±1.023 U/ml (p<0.001) and IAA — 11.05±1.20 U/ml (p<0.05), respectively, we received in the group of DAA-positive children at the late preclinical stage of T1D development which preceded to the clinical debut of disease in the chronological aspect.

The prospective pathogenetic priority of elevated IA-2A and GADA titers was determined comparing to IAA content at the preclinical and early clinical stages of T1D development in DAA+ children and adolescents. The significantly highest titers of IAA, GADA, and IA-2A compared with titers of DAA+ children at early stage of T1D development and DAA levels of children with a clinical debut of disease, respectively, were noted in the group of DAA+ children at late latent stage of T1D development, approximate in time to the clinical debut of disease [24-26]. The reduced frequency detection of DAA and the lower values of their levels were registered by us in children with newly detected T1D compared with the group of DAA+ children at the late preclinical stages of disease development confirms the completion of autoimmune destruction for desensitizing autoantigens, and, as a result, a decrease in previously elevated DAA titers, which is confirmed in recent publications [17, 22-25].

Determining the duration of preclinical stage of type 1 diabetes development by detecting the values of simultaneously raised titers of DAA − IA-2A and GADA. The prognostic significance for the combination of increased IA-2A and GADA titers was identified as the major marker predicting T1D manifestation and significantly predicts the duration of the preclinical stage of disease development. When analyzing the time of clinical debut of T1D in 86 DAA+ patients **(Fig. 1)**, we found that clinical debut of T1D occurred in 43 (50.00%) children within the first 3 years from the moment of the first determination of elevated titers of DAA, the disease manifestation was registered in 19 (22.10%) of patients in the period from 3 to 5 years after inclusion of patients in the group of DAA+ children, in 14 (16.27%) of children the newly onset T1D was diagnosed after 5 years and in 10 (11.63%) − 10 years after the initial simultaneous detection of high IA-2A and GADA values in the blood of DAA+ patients.
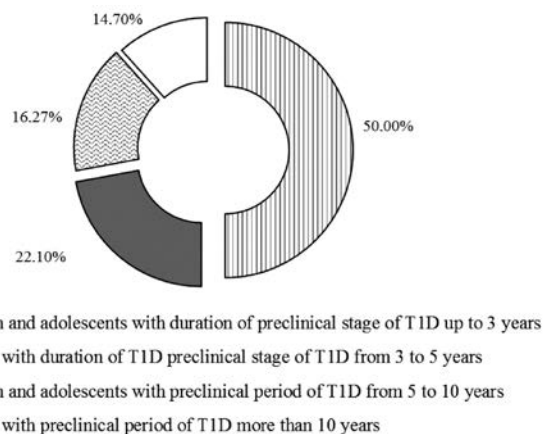

Fig. 1. Clinical manifestation of T1D in DAA+ children depending on the duration of the preclinical stage of disease.

Analyzing the combination and the titer values in the group of DAA+ children with a minimum duration of preclinical stage of T1D up to 3 years, a significant prevalence of tandem combination of IA-2A and GADA with elevated titers was determined as well as at early 7.57±1.14 U/ml; 14.897±2.44 U/ml and at the final stages of the preclinical period of T1D development IA-2A − 19.11±2.48 U/ml; GADA − 18.096±2.71 U/ml, respectively, compared with similar indicators, such as IA-2A − 13.34±1.96 U/ml and GADA − 16.49±2.58 U/ml in the group of patients with

the type 1 diabetes debut **(Fig. 2)** compared with similar values of DAA+ patients with a duration of preclinical stage of T1D from 3 to 5 years (IA-2A − 5.75±1.06 U/ml and GADA − 10.40±1.74 U/ml, p<0.05 and IA-2A − 9.81±2.17 U/ml and 11.71±3.84 U/ml, p<0.05, respectively) compared to similar values, such as IA-2A − 7.78±1.03 U/ml and GADA − 11.05±1.34 U/ml in the group of patients with type 1 diabetes debut **(Fig. 3)** and in patients with newly diagnosed T1D, where the latent stage of disease development lasted from 5 to 10 years (IA-2A − 4.30±1.48 U/ml and GADA − 3.84±1.13 U/ml, p<0.05 and IA-2A − 7.79±3.47 U/ml and GADA − 6.32±1.34 U/ml, p<0.05, respectively) compared to similar values, such as IA-2A − 6.43±2.35 U/ml and GADA − 5.08±1.23 U/ml in the group of patients with type 1 diabetes debut **(Fig. 4)** and in patients with T1D debut and the preclinical period duration of disease formation over 10 years (IA-2A − 3.20±1.13 U/ml, GADA − 2.46±1.21 U/ml, p<0.05, and IA-2A − 5.49±3.03 U/ml, GADA − 4.93±1.76 U/ml, p<0.05, respectively) compared with similar values, such as IA-2A − 4.13±2.16 U/ml and GADA − 3.18±1.57 U/ml in the group of patients with type 1 diabetes mellitus debut **(Fig. 5)**.
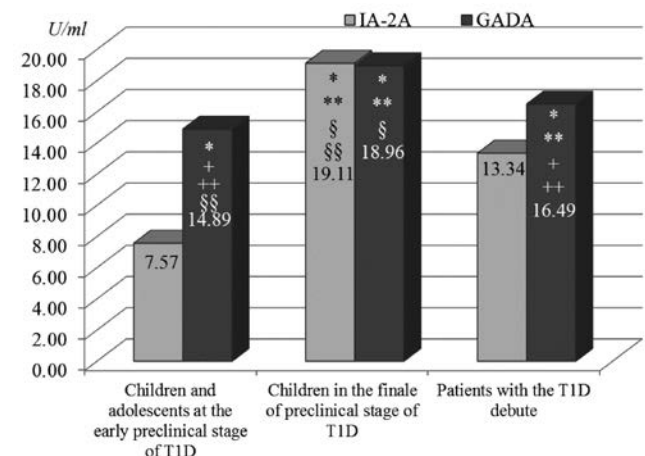

Fig. 2. The dynamics of IA-2A and GADA titers in patients with a duration of the preclinical period of T1D up to 3 years: * — p_1<0.001, compared with IA-2A in group of DAA+ children at early preclinical stage; ** — p_2<0.05, compared with GADA in group of DAA+ children at early preclinical stage; + — p_3<0.001, compared with IA-2A in group of DAA+ children in the finale of the preclinical stage; ++ — p_4<0.001, compared with GADA in group of DAA+ children in the finale of the preclinical stage; § — p_5<0.001, compared with IA-2A in group of DAA+ children with T1D debut; §§ — p_6<0.05, compared with GADA in group of DAA+ children with T1D debut.

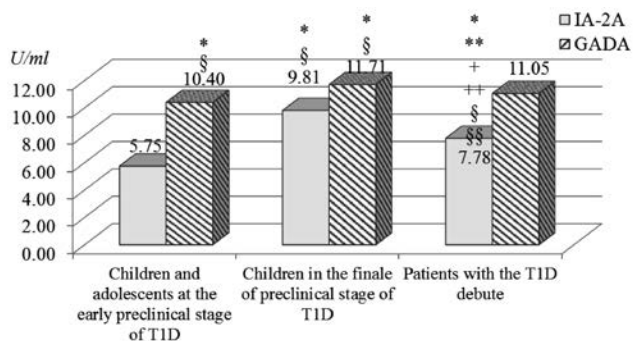VERTE ▶

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Fig. 3. Dynamics of IA-2A and GADA titers in patients with a duration of T1D preclinical stage from 3 to 5 years: * — $p_1<0.001$, compared with IA-2A in group of DAA+ children at early preclinical stage; ** — $p_2<0.05$, compared with GADA in group of DAA+ children at early preclinical stage; + — $p_3<0.05$, compared with IA-2A in DAA+ group of children in the finale of the preclinical stage; ++ — $p_4<0.001$, compared with GADA in group of DAA+ children in the finale of the preclinical stage; § — $p_5<0.001$, compared with IA-2A in group of DAA+ children with T1D debut; §§ — $p_6<0.05$, compared with GADA in group of DAA+ children with T1D debut.
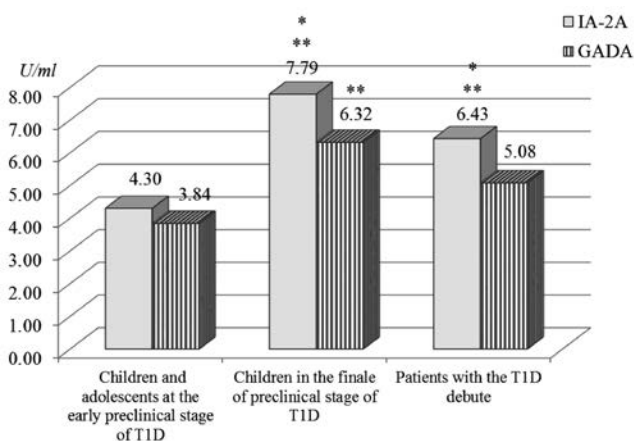
Fig. 4. Dynamics of IA-2A and GADA titers in patients with duration of latent stage of T1D development from 5 to 10 years: * — $p_1<0.001$, compared with IA-2A in group of DAA+ children at early preclinical stage; ** — $p_2<0.05$, compared with GADA in group of DAA+ children at early preclinical stage; + — $p_3<0.05$, compared with IA-2A in group of DAA+ children in the finale of the preclinical stage; ++ — $p_4<0.001$, compared with GADA in group of DAA+ children in the finale of the preclinical stage; § — $p_5<0.001$, compared with IA-2A in group of DAA+ children with T1D debut; §§ — $p_6<0.05$, compared with GADA in group of DAA+ children with T1D debut.

Thus, the highest values of elevated IA-2A and GADA titers were determined in the group of DAA+ children both at baseline and in the final of the preclinical stage of T1D development, and the maximum increase of IA-2A and GADA titers was detected in the finale of preclinical stage of T1D development in DAA+ children and adolescents in whom the disease manifestation
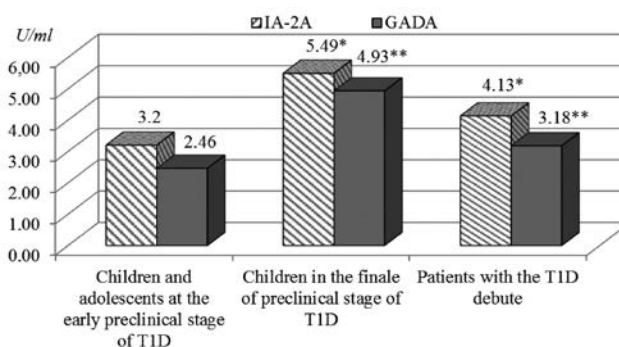

Fig. 5. Dynamics of IA-2A and GADA titers in patients with the duration of latent stage of T1D development more than 10 years: * — $p_1<0.05$, compared with IA-2A in group of DAA+ children at early preclinical stage; ** — $p_2<0.05$, compared with GADA in group of DAA+ children at early preclinical stage.

occurred within the first three years after the establishing DAA-positive status, which allows us quite accurately predict the time of clinical debut of T1D in DAA+ children [26]. Thus, it was found that the prevalence of tandem-simultaneous combination increasing the autoantibody titers — IA-2A and GADA has a pathogenetic priority importance during the evolution of T1D latent stage, which is consistent with the data of other authors [16, 23, 27-29].

Dysglycemia at the early and late preclinical stages of T1D development. Recently, there were appeared new data regarding that IA-2A and GADA are not only absolutely significant predictors of T1D development, but also serve as accurate markers of impaired carbohydrate metabolism preceding the clinical debut of disease [30-32]. In studies of other authors, it was found that the progressive production of diabetes-associated autoantibodies to islet autoantigenes correlates with a rapid loss of beta-cell function [33-35].

The criterion for inclusion in the group of patients with dysglycemia were impairments of fasting glycemia >6.1 mmol/L, borderline hyperglycemia at the end of 2 hour point when performing OGTT in the range of 7.8-11.1 mmol/L, or determining the integration index — HbAlc>6.1%, since the HbA1c level shows at the same time both fasting, preprandial and postprandial glucose contents. These examinations and observations made it possible to identify four groups of patients **(Table 1)**. HbAlc values, basal glycemia, postprandial glycemia in OGTT were studied by us in each of the examined groups. The largest group (group 4) consisted of DAA– children with

Table 1. Indicators of basal and postprandial glycemia (mmol/L) at the 2-hour point in OGTT of DAA+ and DAA- children (M±m)

Group DAA+ or DAA-	Basal glycemia mmol/L	Glycemia at the 2-hour point in OGTT	The total number of children in the group	Percentage of total number of children
1 DAA+	6.31±0.28$^{+\#}$	9.8±0.31$^{*+\#\S}$	25	5.47%
2 DAA+	4.80±0.46§	5.1±0.03$^{\#\S}$	125	27.35%
3 DAA+	5.03±0.51	7.92±0.04$^{*+\#\S}$	12	2.62%
4 DAA-	4.99±0.67	4.54±0.11$^{\#\S}$	295	64.55%

Notes: * — $p_1 < 0.001$, compared with group of DAA– children (group 4); $^+$ — $p_2 < 0.05$, compared with group of DAA+ children at early preclinical stage of T1D development (group 2); $^\#$ — $p_3 < 0.05$, compared with group of DAA+ children at early preclinical stage of T1D development (group 3); § — $p_4 < 0.05$, compared with group of DAA+ children at late latent stage of T1D development (group 1).

no signs of chronic hyperglycemia (HbAlc<6.1%) (Table 1). This group included 295 (64.55%) examined DAA– children; Group I — 25 DAA+ patients with signs of chronic hyperglycemia (HbAlc>6.1%) without clinical symptoms of T1D, which amounted up to 5.47%. T1D was debuted in all 25 children of this group for the period from 6 months to 3 years, which confirms the diagnostic value of the combined detection of immunological markers of autoimmune β-cell destruction and metabolic markers of impaired carbohydrate metabolism — impaired fasting glycemia (glucose) and postprandial glycemia (glucose) at 2-hour point in OGTT. Group 2 included DAA+ children without signs of impaired carbohydrate metabolism (HbAlc<6.1) — 125 (20.76%) children. During the process of dynamic observation of these patients for 10 years and with the repeated determination of DAA in the blood of 49 (45.12%) children of this group, the clinical debut of T1D was diagnosed. OGTT was identified as normal in the group II of DAA+ children with HbAlc<6.1 and without dysglycemia. Over time, group 3 of 12 DAA+ children with HbAlc<6.1 were retrospectively identified from the group 2 of DAA+ children, in which T1D was developed after 4-5 years (2.62%) (**Fig. 6**).

When performing OGTT in DAA+ children of this group, dysglycemia in the form of borderline hyperglycemia at the end of the 2-hour point was found — 7.92±0.04 mmol/L compared to normal basal glycemia values 5.03±0.51 mmol/L (p<0.001), which allowed to retrospectively identify DAA+ children of this group as being in the early preclinical period of T1D development.
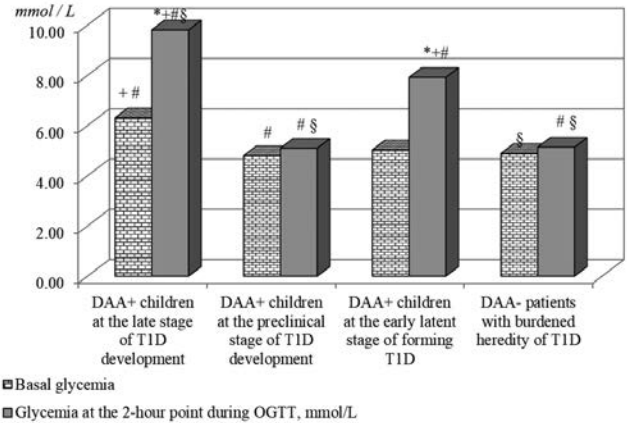

Fig. 6. Basal and postprandial glycemia at the 2-hour point when performing OGTT with glucose load in DAA+ and DAA- children (mmol/L): * — $p_1 < 0.001$, compared with group of DAA+ children with analogous values; $^+$ — $p_2 < 0.05$, compared with group of DAA+ children at preclinical stage of T1D development with analogous values; $^\#$ — $p_3 < 0.05$, compared with group of DAA+ children at early latent stage of T1D development with analogous values; § — $p_4 < 0.001$, compared with group of DAA+ children at the late latent stage of T1D development with analogous values.

In 295 (74.32%) DAA- children of group 4, HbAlc values were <6.1 and no symptoms of dysglycemia were recorded in any of its manifestations. The change in DAA titer by the moment of hyperglycemia onset was unidirectional in nature in all the children: autoantibodies to islet antigens were determined in increasing titer values.

Thus, it was found that the early stage of the preclinical period of T1D development is characterized by the presence of dysglycemia at the 2-hour point in 2.62% DAA+ children during the OGTT implementation. For some DAA+ children (5.47%), who were at the late stage of the preclinical period of T1D formation is characterized as well as the presence of postprandial hyperglycemia in OGTT — 9.8±0.31 mmol/L, and dysglycemia in the form of impaired fasting glycemia — 6.31±0.28 mmol/L (p<0.001).

The content of C-peptide in DAA+ children at different stages of T1D development. Our studies have shown that there is a significant decrease in both basal and stimulated secretions of C-peptide in the group of DAA+ children, under the development of dysglycemia in the form of impaired postprandial glycemia at 2-hour point in OGTT, and impaired fasting glycemia (**Table 2**).

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Table 2. Content of basal and stimulated C-peptide (ng/ml) in DAA+ and DAA- children at different stages of T1D development (M±m)

Group	DAA+ or DAA-	Basal C-peptide, ng/ml	Stimulated C-peptide, ng/ml
1	DAA+ (n=25)	0.21±0.01*+#	0.45±0.08*+#
2	DAA+ (n=125)	0.92±0.02*#	2.15±0.04*#+
3	DAA+ (n=12)	0.83±0.05*§	1.13±0.06*+§
4	DAA- (n=295)	1.24±0.04	3.12±0.06*

Notes: * — $p_1 < 0.001$, compared with group of DAA+ children with analogous values (group 4); + — $p_2 < 0.05$, compared with group of DAA+ children at preclinical stage of T1D development with analogous values (group 2); # — $p_3 < 0.05$, compared with group of DAA+ children at early latent stage of T1D development (group 3); § — $p_4 < 0.05$, compared with group of DAA+ children at the late latent stage of T1D development with analogous values (group 1).

In all DAA+ children in this group, T1D was debuted within 3 years. In this group of children, the values of basal C-peptide secretion were recorded at reduced levels long before T1D debut (Table 2).

Further studies showed that in the group DAA+ children, with no metabolic changes in carbohydrate metabolism, HbAlc<6.1%, the average content of basal C-peptide was determined at the level of 0.92±0.02 ng/ml, and stimulated ones — at the level of 2.15±0.04 ng/ml (p<0.001), which indicated its preservation. In group 3 of DAA+ children, at the early latent stage of T1D development, with HbAlc<6.1 and dysglycemia in the form of borderline hyperglycemia at the end 2-hour point in OGTT and with T1D debut during the next 4-5 years, the average content of basal C-peptide was determined at the level of 0.83±0.05 ng/ml, stimulated ones — at the level of 1.13±0.06 ng/ml (p<0.05), which indicated a slight decrease in β-cell secretory capacity. In DAA-children of group 4 with HbAlc<6.1 and without dysglycemia in any manifestation, the secretory activity of β-cells was completely preserved, with the basal value being 1.24±0.04 ng/ml and stimulated — 3,120,06 ng/ml (p<0,05) secretions of C-peptide showed complete preservation of secretory capacity of insulin-producing pancreatic β-cells and were almost completely consonant with basal 1,29±0,05 ng/ml and stimulated 3.94±0.09 ng/ml (p<0.001) numbers of C-peptide secretion in children of control group (**Fig. 7**).

In the group of children with newly diagnosed T1D, the concentration of basal C-peptide in the blood was lower than the norm — 0.31±0.02 ng/ml, and the reserves of stimulated
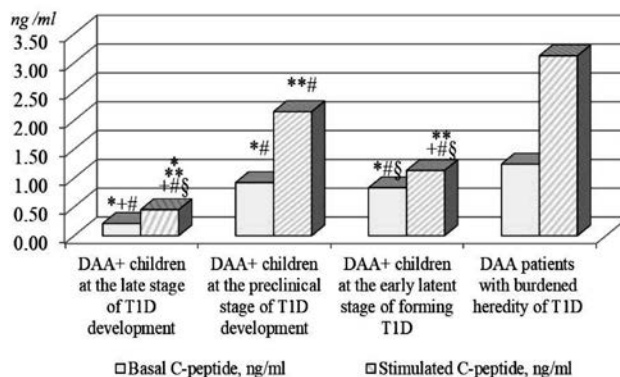

Fig. 7. Content of basal and stimulated C-peptide in DAA+ and DAA- children at different stages of T1D development (ng/ml): * — $p_1 < 0.001$, compared with group of DAA+ children with analogous values; + — $p_3 < 0.001$, compared with group of DAA+ children at early preclinical stage of T1D development with analogous values; # — $p_4 < 0.05$, compared with group of DAA+ children at the early latent stage of T1D development with analogous values; § — $p_5 < 0.05$, compared with group of DAA+ children at early latent stage of T1D development with analogous values.

C-peptide were practically exhausted — 0.49±0.06 ng/ml (p<0.05), which is absolutely pathognomonic for the clinical debut of T1D. Thus, the performed studies revealed a significant secretory range in the C-peptide content at the stages of progressive development of T1D. The secretory capacity of DAA+ children with metabolic markers of carbohydrate metabolism disorders were saved, but there is a tendency for their decline. Conversely, the results of our studies on the C-peptide content day before the debut and in the disease manifestation, make the fact indisputable and coincide with the data of other authors affirming that significantly reduced the C-peptide level — the result of prolonged autoimmune aggression aggression in time, which is ending allowing us to use the study of C-peptide level together with DAA, as a hormonal marker of T1D development in children and adolescents [21, 36].

Levels of different types of cytokines (IL-1, IL-4, IL-6, IL-10, IFNγ, TNFα, IL-8, IL-16) in children blood at the preclinical and early clinical stages of T1D development. *IL-1*. A study of IL-1α level in PB of DAA+ children revealed that the median (Me) of its content (9.16; 5.00-17.5 pg/ml) (**Table 3**) was more than twice that in DAA- children and children of control group. In children with T1D debut Me of IL-1α content in the PB was also slightly elevated, but not to the extent that DAA+

children had. The content of IL-1β in the PB serum was practically undetermined in all 4 groups of children examined. A more significant increase in the level of circulating IL-1α at the preclinical stage compared to the clinical stage of T1D may be explained by the fact that the most active autoimmune process in IL occurs at the latent stage. As most β-cells are destructed, the autoimmune process gradually subsides, which is reflected in the reduction of IL-1 levels in many patients with already debuted T1D. M.J. Hussain et al. [37] when determining the IL-1α content in children with newly diagnosed T1D a significant increase in its level in PB revealed. Higher IL-1α levels were also observed in the pre-diabetes period in identical twins. In addition, children with diabetes and their sibs had a decrease in the IL-1α production by PB mononuclear cells after their stimulation by mitogens in vitro. No such changes were observed in T1D with a long course and in T2D. It was somewhat surprising that IL-1α, which has the most pronounced cytotoxic effect on IL in vitro, was not detected at all in the PB of the examined subjects.

Our studies of IL-1α and IL-1β in children with burned heredity at preclinical and early clinical periods of T1D development confirm the data of M.J. Hussain [37], that there is a significant increase in the level of IL-1α and not of IL-1β in the PB at the latent and clinical stages of T1D. This opinion is confirmed by recent publications [38], which found similar changes in the level of IL-1α and IL-1β in the PB of healthy children and children with T1D.

IL-4. The content of IL-4 cytokine in the PB of children from control group ranged from 0 to 7.7 pg/ml (Me – 0.55; 00-3.00 pg/ml) (Table 3). Unlike DAA-, the IL-4 content in the PB iof DAA+ children was not determined (Me – 0; 0.00-0.00 pg/ml), which made a high significant difference for the group of DAA- children (p<0.001). The data obtained by us are confirmation of the point of view of other authors [39], that IL-4 plays a protective role in the human body by participating in mechanisms that impede the T1D development. In contrast to proinflammatory cytokines, the content of which is increased at different stages the autoimmune process, the level of IL-4 is sharply reduced and its overall decrease is often recorded, practically at zero values. Therefore, the practical absence of IL-4 cytokine in circulation of DAA+ children may serve as a marker that indicates the possibility of rapid development of the clinical debut of disease and provides an opportunity to conclude that there is a presence of preclinical stage of T1D development.

IL-6. The level of proinflammatory cytokine IL-6 in most DAA+ children (Table 3) was more elevated (fluctuations from 1.0 to 174.0 pg/ml) in the PB compared to DAA- children and those of control group, that coincides with data [38]. Moreover, in 10 DAA+ children, the IL-6 level in PB reached the significant values (from 11.00 to 174.00 pg/ml), and in three of them with particularly high levels of IL-6 in the PB (18.0; 22.0 and 174.0 pg ml) over time a clinical debut of T1D with a more aggressive course than in other children with this disease was diagnosed. It is also important to emphasize that in three children with T1D, and quite high level of IL-6 in the PB (84.0-95.0 pg/ml), complications of inflammatory nature were appeared after 5-7 years. Thus, the data obtained demonstrate that the peak of the highest level of IL-6 in circulation was detected at the latent stage long before the clinical signs of the disease, which

Table 3. The level of different types of cytokines in the blood of children at preclinical and early clinical stages of T1D development (pg/ml)

Type of cyto-kines	Healthy children (n=155)	DAA- children (n=295)	DAA+ children (n=162)	DAA+ children with T1D debut (n=86)
IL-1α, Me	3.7 (0.00-6.00)	2.85* (0.00-5.40)	9.16* ** (5.00-17.5)	4.00 (2.00-19.00)
IL-4, Me	0.55 (0.00-3.00)	0.00* (0.00-0.00)	0.00* ** (0.00-0.00)	0.00* (0.00-0.00)
IL-6, Me	0.00 (0.00-1.06)	1.50 (0.00-4.00)	2.50* ** (1.60-11.00)	1.00+ (0.60-2.00)
IL-10, Me	2.10 (0.50-3.00)	2.60 (1.40-5.50)	2.00* ** (1.50-5.00)	2.00 (1.50-3.00)
IFNγ, Me	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
TNFα, Me	0.75 (0.00-1.00)	2.00* (0.00-4.00)	3.50* (0.50-6.50)	1.55** (0.00-3.00)
IL-8, Me	0.00 (0.00-3.70)	0.00 (0.00-0.00)	23.00* ** (0.00-30.00)	6.50** (0.00-10.50)
IL-16, M±m	136.3±19.1	153.4±16.2	197.3±16.3* **	73.2±9.7* **+ **

*Notes: * — p_1<0.05 compared with control group of children; ** — p_2<0.05 compared with group of DAA- children; + — p_3<0.05 compared with group of DAA+ children.*

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is a reflection of the maximum intensity of the autoimmune process in IL and are confirmed by the work of a number of authors [40, 41].

IL-10. It was shown (Table 3) that Me of the IL-10 content in PB in all groups of examined children was not significantly different, although it was slightly lower in healthy, DAA- children and children with T1D compared to DAA+. However, in three DAA+ children the level of this cytokine in circulation ranged from 5.0 to 11.0 pg / ml, and in three patients with T1D — from 5.0 to 8.5 pg/ml. The results obtained are consistent with those of other authors [42], which in some children with T1D sometimes found increased levels of circulating IL-10. This has been confirmed in recent publications [38]. At the same time, it has been shown that mononuclear cells isolated from the PB of patients with T1D secrete less number of IL-10 than mononuclear cells of healthy individuals and that when incubated IL-10 with human IL in vitro, it has the ability to protect them from the destructive action of proinflammatory cytokines [43].

IFNγ. No significant changes in IFNγ content in PB of DAA+ children and patients with T1D were detected. In all four groups of examined children (Table 3), Me of IFNγ content in PB was zero. However, in 4 of DAA+ children, the increased level of IFNγ in PB ranged from 6.0 to 21.0 pg/ml. Its content was slightly increased in the PB (from 2.0 to 5.0 pg/ml), close to the possibilities of the method sensitivity was in 3 patients with T1D. According to the available data, IFNγ, especially in combination with IL-1β and TNFα, causes a pronounced cytotoxic effect on human IL in vitro in tissue culture [43]. At the same time, the data on the level of IFNγ in PB of patients with T1D are extremely controversial. Some authors [44, 39] even report of a decrease of IFNγ content in PB of patients with T1D debut and in DAA+ children. However, recent publications indicate a devastating increase in IFNγ levels at the early clinical period of T1D development [45]. Thus, it can be assumed that the local increase of IFNγ content in IL does not exclude the simultaneous decrease of its concentration in PB. The increase in IFNγ content in PB was observed of some children at preclinical stage and T1D debut may be due not only to the autoimmune process in IL but also to other causes, in particular, manifest and early development of T1D complications.

TNFα. It was shown that Me of the content of cytokine TNFα in PB of DAA+ children significantly exceeds that of DAA- and children of control group (Table 3). In children with already manifested T1D, there was a decrease in Me of TNFα content compared with DAA+ children, although in 9 children it was elevated and in 3 of them it was 11.0-15 pg/ml, which is consistent with the data of other authors [38]. Thus, the obtained results indicate that in many children with the preclinical stage of T1D, an increased level of TNFα in PB is characteristic, reflecting the degree of aggressiveness of autoimmune destruction of β-cells in IL at the preclinical stage of T1D development. In some children with advanced disease, the decrease of TNFα in PB is explained by a decrease in autoimmune aggression [46].

IL-8. Studying proinflammatory α-chemokine IL-8 (Table 3) a marked increase of its content in PB was revealed in DAA+ children. Thus, in the 8 examined patients, IL-8 levels in the PB exceeded 20 pg/ml, whereas among DAA- children, IL-8 was detected in PB only in one child. In the control group of children, the content of IL-8 ranged from 1.6 to 10 pg/ml. In children with newly diagnosed T1D, the increase of IL-8 content in PB was in 6 children, Me of the content was 6.5 pg/ml, ie was significantly less than in DAA+ children. The data obtained are consistent with the limited data on this issue [47, 48], that the elevated level of IL-8 in PB is characteristic for many patients at the initial stage of T1D. At the same time, the data indicate that the highest content of chemoattractant IL-8 in PB is observed not only, but also in such purely inflammatory cytokines as IL-6 and TNFα at the latent stage of T1D development. This it allows us to predict that IL-8 is involved in the migration of autoreactive cells at the earliest stages of the autoimmune process development long before the clinical manifestation of disease, which promotes the migration of autoreactive lymphocytes from the blood to the focus of inflammation, that is, still in a practically «healthy» child. As the destruction of β-cells is finishing, a decrease of IL-8 level in circulation is noted.

IL-16. Pronounced statistically significant increase of IL-16 level was shown in the PB of DAA+ children (Table 3). In already developed T1D, the average content of IL-16 in the PB was reduced. The results obtained suggest that

at the latent stage of T1D development, that is, during the period of the most active destruction of β-cells, IL-16, like other types of chemokines, promotes the migration of autoreactive T cells in IL, where they, through proinflammatory cytokines, carry out the destruction and apoptosis of insulin-producing cells. When the intensity of the autoimmune process decreases, the concentration of IL-16 in the PB decreases below the norma and, accordingly, the chemotaxis of effector mononuclear cells in IL is weakened or terminated. Thus, for the first time it has been shown that IL-16, like other types of typical chemokines, is actively involved in the earliest stage of the autoimmune process development in IL, providing a foci of inflammation by antigen-dependent cells. As the inflammatory process attenuates, IL-16 level in the PB decreases as there is no need for its stimulating high content in blood circulation due to the total death of most β-cells. Thus, as can be seen from the above mentioned, the studies confirm the data of other authors that the level of most types of cytokines in the PB of both healthy and with T1D child has significant individual fluctuations caused by different biological purpose of different types of cytokines, their local and remote actions, as well as th complex relationships in the «cytokine network», i.e. when the neutralization is possible on the periphery or, conversely, an enhancement of the action of certain types of cytokines [2, 37]. At the same time, the studies have shown that many normoglycemic children with burdened heredity at the preclinical period of T1D development have a clear increase in such proinflammatory cytokines as IL-1α, IL-6 and TNFα in the PB, which are markers of inflammation [49], and the decrease in the level of the anti-inflammatory cytokine IL-4 involved in the protective mechanism for T1D [39]. In children with already clinically manifested T1D, a similar imbalance of cytokine content in the PB was observed, but the degree of its maifesttation was less significant. The level of cytokines and chemokines in the PB of children with T1D is different depending on the dynamics of disease development. At the preclinical period, when the child is still «practically healthy», the most significant disturbance of the cytokine level occurs, reflecting the peak of autoimmune aggression, which attenuates as the destruction

of most β-cells, which leads to hypoinsulinemia and, therefore, is a reflection of the highest degree of autoimmune process intensity which results in total destruction of β-cells. The detected changes from the proinflammatory cytokines are consistent with the dynamics of changes in the level of chemoattractants IL-8 and IL-16, which carry out migration of autoreactive cells from circulation to IL, i.e., a sharp increase in the content of IL-8, IL-16 number at the latent period of T1D development and decrease of chemoattractant concentrations in the disease manifestation, which is confirmed by the works of other authors, in particular M. Baggiolini [50], who showed that the increase in chemokine levels occurs only at the preclinical stage of T1D development. The data obtained are also in line with the results of recent studies, which, using FACS analysis, revealed similar dynamic changes in the number of CD3 +, CD4 +, CD8 + T lymphocytes in the PB which are cells-producers of proinflammatory cytokines [51] in DAAb-positive children at preclinical and early clinical periods of disease [2]. These results may be useful in the development of novel specifically targeted methods of therapy and prophylaxis for T1D that block certain links of immune system involved, mainly through cytokines, in the destruction of insulin-producing cells.

Conclusions

1. It was found that the increased titer of DAA in 162 (35.45%) of 457 children with burdened heredity for not less than twofold determination of presence in them diabetes-associated autoantibodies, mainly GADA and IA-2A was noted, clinical debut of T1D was manifested in 86 (72.34%) of them in the period from 6 months to 16 years (27.4±4.3 months).
2. It is determined that the most significant markers of T1D development are antibodies to the protein tyrosine phosphatase and antibodies to decarboxylase glutamic acid, which is confirmed by both the prevailing increase in the titers of IA-2A and GADA, and the dominant frequency of their combined occurrence.
3. In children with faster clinical manifestation of T1D and duration of preclinical stage of disease development less than 3 years, the prevalence

of values of elevated titers of IA-2A and GADA is detected at both the early and final stages of the preclinical period of T1D development compared with similar indicators in patients with duration of T1D preclinical stage from 3 to 5 years and in patients with newly diagnosed T1D in which the latent stage of disease development lasted more than 5 and 10 years.

4. Analysis of the time of T1D manifestation in 86 patients with the presence of high titers of DAA established in 50.00% of them the clinical debut of T1D, which occurred within 3 years from the moment of the first determination of the increased titers of DAA, in 22.10% the onset of the disease was diagnosed in terms from 3 to 5 years after the inclusion of patients in the group of DAA+ children, in 16.27% the first diagnosed T1D was in 5 years after the initial detection of simultaneously increased titers of two from the three types of studied DAA in the blood of patients and in 11.63% of DAA+ children — in 10 years after the primary simultaneous detection of high values of IA-2A and GADA in the blood of DAA+ patients.

5. The early preclinical period of T1D development in children with the presence of increased titers of DAA was characterized by the presence of dysglycemia in the form of increased glycemia in 2 hours after the glucose tolerance test and a slight decrease in the secretion of stimulated C-peptide; in addition, in late preclinical period in DAA+ children dysglycemia was developed in the form of impaired fasting glycemia, as well as a decrease in both basal and stimulated secretion of C-peptide, indicating gradually decrease of potential secretory capacity of insulin-producing β-cells of the Langerhans islands at preclinical period of T1D development.

6. Development of T1D in DAA+ children is preceded by an increase in the level of proinflammatory cytokines IL-1α, IL-6 and TNFα, chemokines IL-8 and chemoattractant IL-16, which positively correlates with increased titers of IA-2A and GADA, while reducing IL concentration in the PB was observed. These changes in indicators of immunity were less pronounced after the clinical debut of disease, confirming the pathogenetic key role of cytokines in the preclinical period of T1D pathogenesis.

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Алгоритм передклінічної діагностики цукрового діабету 1-го типу як підґрунтя для створення Реєстру ДААт-позитивних дітей і підлітків України з прогнозованим розвитком захворювання

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Резюме. Мета — встановлення механізмів розвитку цукрового діабету 1-го типу (ЦД1) на ранній і пізній передклінічних стадіях формування захворювання в дітей і підлітків. **Матеріал і методи.** У ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України» було ініційовано програму «Імунітет у передклінічний період розвитку ЦД1», на базі якої створено Реєстр маркерпозитивних дітей із прогнозованим розвитком ЦД1, що налічує 612 дітей віком від 7 до 15 років з обтяженою спадковістю, в яких визначено титр діабетасоційованих автоантитіл (ДААт), цитокінів, базальної та постпрандіальної глікемії й секреції С-пептиду на передклінічному та клінічних етапах розвитку ЦД1. **Результати.** Отримано нові дані, які дозволили суттєво доповнити існуючі уявлення про патогенез ЦД1. У результаті виконаного дослідження сформовано групу маркерпозитивних дітей з обтяженою спадковістю та прогнозованим ризиком розвитку захворювання. У 162 (35,45%) із 457 дітей з обтяженою спадковістю після щонайменше дворазового визначення ДААт виявлено їх підвищений титр, переважно GADA та IA-2A, у 86 (53,08%) із них у термін від 6 міс. до 16 років (27,4±4,3 міс.) маніфестував ЦД1. Визначено формулу поєднаної наявності та значень одночасно підвищених титрів ДААт до острівцевих автоантигенів, а саме IA-2A+GADA, що є предиктором як тривалості передклінічної стадії розвитку ЦД1, так і швидкості дебюту. Встановлено також порушення продукції цитокінів (підвищення рівня прозапальних цитокінів ІЛ-1α, ІЛ-6 і ЧНПα, ІЛ-8 і ІЛ-16 з одночасним зниженням концентрації ІЛ-4

Оригінальні дослідження

у крові) як ключових чинників патогенезу ЦД1, що зумовлюють як швидкість дебюту ЦД1, так і агресивність його перебігу. Встановлено, що ранній передклінічний період розвитку ЦД1 у ДААт+ дітей характеризувався наявністю дисглікемії у вигляді підвищеної глікемії через 2 год після виконання тесту толерантності до глюкози та нерізким зниженням секреції стимульованого С-пептиду; крім того, у пізній передклінічний період у ДААт+ дітей приєднувалася дисглікемія у вигляді порушеної глікемії натще, а також знижувалась як базальна, так і стимульована секреція С-пептиду, що свідчить про виснаження потенційних можливостей β-клітин підшлункової залози. **Висновки.** На підставі виконаного клініко-імунологічного дослідження отримано нові дані про механізми розвитку ЦД1 на ранній і пізній передклінічних стадіях формування захворювання в дітей і підлітків, а саме: суттєві зміни вродженого та набутого імунітету, зсуви імунних, метаболічних і гормональних показників як на ранній, так і на пізніших передклінічних стадіях розвитку ЦД1. Ці зміни свідчать, що період хронологічно зростаючих послідовних поломок у різних ланках імунітету значно випереджає час клінічного дебюту ЦД1, і можуть бути використаними як предиктори розвитку захворювання. Створено Реєстр маркерпозитивних дітей із прогнозованим розвитком ЦД1.

Ключові слова: цукровий діабет 1-го типу, діти та підлітки, діабетасоційовані автоантитіла (ДААт), автоантитіла до декарбоксилази глютамінової кислоти (GADA), автоантитіла до протеїну тирозинфосфатази (IA-2A), цитокіни, базальна та постпрандіальна глікемія, базальний і стимульований С-пептид.

Алгоритм доклинической диагностики сахарного диабета 1-го типа как основа для создания Реестра ДААт-позитивных детей и подростков Украины с прогнозированным развитием заболевания

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Резюме. Цель — установление механизмов развития сахарного диабета 1-го типа (СД1) на ранней и поздней доклинических стадиях формирования заболевания у детей и подростков. **Материал и методы.** В ГУ «Институт эндокринологии и обмена веществ им. В.П. Комиссаренко НАМН Украины» инициирована программа «Иммунитет в доклинический период развития СД1», на базе которой создан Реестр маркерпозитивных детей с прогнозированным развитием СД1, который насчитывает 612 детей в возрасте от 7 до 15 лет с отягощенной наследственностью по СД, у которых определен титр диабетассоциированных аутоантител (ДААт), уровней цитокинов, показателей базальной и постпрандиаль-

ной гликемии и секреции С-пептида на доклиническом и клинических этапах развития СД1. **Результаты.** Получены новые данные, которые позволили существенно дополнить существующие представления о патогенезе СД1. В результате выполненного исследования сформировано группу маркерпозитивных детей с отягощенной наследственностью и прогнозированным развитием заболевания. У 162 (35,45%) из 457 детей с отягощенной наследственностью после не менее чем двухкратного определении ДААт выявлен повышенный титр ДААт, преимущественно GADA и IA-2A, у 86 (53,08%) из них в течение периода от 6 мес. до 16 лет (27,4±4,3 мес.) манифестировал СД1. Определена формула сочетанной встречаемости и значений одновременно повышенных титров ДААт к островковым аутоантигенам, а именно IA-2A+GADA, которые являются предиктороми как длительности доклинической стадии развития СД1, так и скорости дебюта. Установлено также нарушение продукции цитокинов (повышение уровня провоспалительных цитокинов ИЛ-1α, ИЛ-6 и ФНОα, ИЛ-8 и ИЛ-16 при одновременном снижении концентрации ИЛ-4 в крови) как ключевых факторов патогенеза СД1, которые обусловливают как скорость дебюта СД1, так и агрессивность его течения. Установлено, что ранний доклинический период развития СД1 у ДААт– детей характеризуется наличием дисгликемии в виде повышения гликемии через 2 часа теста толерантности к глюкозе и нерезким снижением стимулированной секреции С-пептида; в поздний же доклинический период у ДААт+ детей дополнительно развивалась дисгликемия в виде нарушенной гликемии натощак, а также снижались как базальная, так и стимулированная секреция С-пептида, что свидетельствует о постепенно нарастающем снижении потенциальных секреторных возможностей β-клеток островков Лангерганса в доклинический период развития СД1. **Выводы.** Получены новые данные о механизмах развития СД1 на ранней и поздней доклинических стадиях формирования заболевания у детей и подростков, а именно: выявлены существенные изменения естественного и приобретенного иммунитета, иммунных, метаболических и гормональных показателей как на ранней, так и на более поздних доклинических стадиях развития СД1, которые свидетельствуют о том, что период хронологически нарастающих последовательных поломок в разных цепях иммунитета значительно опережает время клинического дебюта СД1, что может быть использовано в качестве предикторов развития заболевания. Создан Реестр маркерпозитивных детей с прогнозированным развитием СД1.

Ключевые слова: сахарный диабет 1-го типа, дети и подростки, диабетассоциированные аутоантитела (ДААт), аутоантитела к декарбоксилазе глутаминовой кислоты (GADA), аутоантитела к протеину тирозинфосфатазы (IA-2A), цитокины, базальная и постпрандиальная гликемия, базальный и стимулированный С-пептид.