КЛИНИЧЕСКИЕ И ИММУНОЛОГИЧЕСКИЕ ОСОБЕННОСТИ САХАРНОГО ДИАБЕТА У ПАЦИЕНТОВ С АУТОИММУННЫМ ПОЛИГЛАНДУЛЯРНЫМ СИНДРОМОМ 1 ТИПА В РОССИИ

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ОБОСНОВАНИЕ. Аутоиммунный полигландулярный синдром 1 типа (АПС 1 типа) – редкое наследственное аутоиммунное заболевание, возникающее в результате мутаций в гене аутоиммунного регулятора (*AIRE*) и характеризующееся полиорганной дисфункцией. Сахарный диабет (СД) является одним из компонентов этого заболевания.

ЦЕЛЬ. Определение частоты СД среди пациентов с АПС 1 типа в России, выявление клинических и иммунологических особенностей его течения.

МЕТОДЫ. В исследование были включены 113 пациентов с АПС 1 типа, 16 человек из которых имели нарушения углеводного обмена. Исследование антител к глутаматдекарбоксилазе (GAD), цинковому транспортеру-8 (ZnT8), тирозинфосфатазе (IA2), инсулину (IAA) и островковым клеткам поджелудочной железы (ICA) было проведено 30 пациентам с АПС 1 типа без СД и 11 пациентам с АПС 1 типа и СД. Исследование уровня антител проводилось при помощи иммуноферментного анализа.

РЕЗУЛЬТАТЫ. Частота СД в группе пациентов с АПС 1 типа в России составила 14,1% (16/113). Медленно-прогрессирующее течение СД имели 19% пациентов (3/16). При исследовании отмечено, что методы исследования антител к IAA и ICA обладают низкой специфичностью и чувствительностью для диагностики СД при АПС 1 типа. Методы исследования антител к IA2 и ZnT8 обладают высокой специфичностью (100% и 97%), но низкой чувствительностью (42% и 33,3%). Метод определения антител к GAD менее специфичен (70%), а чувствительность его также низкая (58,3%).

ЗАКЛЮЧЕНИЕ. Частота СД у пациентов с АПС 1 типа в России высокая по сравнению с данными авторов из других стран. Около 20% пациентов с АПС 1 типа и СД в России имеют медленно-прогрессирующее течение. Наибольшей специфичностью по отношению к СД у пациентов с АПС 1 типа обладают антитела к IA2 и ZnT8, однако чувствительность их низкая.

КЛЮЧЕВЫЕ СЛОВА: аутоиммунный полигландулярный синдром 1 типа; *AIRE*; сахарный диабет; антитела; глутаматдекарбоксилаза; тирозинфосфатаза; цинковый транспортер-8; инсулин; β-клетки поджелудочной железы

CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF DIABETES MELLITUS IN PATIENTS WITH AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1 IN RUSSIA

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BACKGROUND. Autoimmune polyglandular syndrome type 1 (APS type 1) is a rare inherited autoimmune disease caused by mutations in *AIRE* gene (autoimmune regulator) and characterized by list of components. Diabetes mellitus (DM) can be one of components of this disease.

AIMS. To show frequency of DM in patients with APS type 1 in Russia, to describe clinical and immunological aspects of DM in patients with APS type 1

MATERIALS AND METHODS. 113 patients have been enrolled in the study, 16 of them had DM (15/16) or impaired glucose tolerance (1/16). Antibodies against glutamate decarboxylase, tyrosine phosphatase, zinc transporter-8, insulin and β-cells of pancreas were investigated in 30 patients with APS type 1 without DM and in 11 patients with APS type 1 and DM. ELISA test was used for detection autoantibodies.

RESULTS. Frequency of DM in patients with APS type 1 in Russia is 14.1% (16/113). Some patients had slow-progressive DM – 19%(3/16). Antibodies against insulin and β-cells were not specific and also were not sensitive markers for DM in APS type 1.

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Antibodies against tyrosine phosphatase and zinc transporter-8 test showed high specificity (100% и 97%), but low sensitivity (42% и 33,3%). Antibodies against glutamate decarboxylase were less specific (70%) and had very low sensitivity (58,3%).

CONCLUSIONS. Frequency of DM in patients with APS type 1 in Russia is high to compare to other countries. 20% of Russian patients had slow-progressive course of DM. Antibodies against tyrosine phosphatase and zinc transporter-8 were the most specific for DM in patients with APS type 1, but sensitivity of these antibodies was low.

KEYWORDS: autoimmune polyglandular syndrome type 1; *AIRE*; diabetes mellitus, antibodies, glutamate decarboxylase; tyrosine phosphatase; zinc transporter-8; insulin; β-cells of pancreas

Autoimmune poly-glandular syndrome type 1 (APS-1) is a rare hereditary (autosomal dominant) disorder characterised by multi-organ autoimmune lesions. APS-1 is associated with defects in the autoimmune regulator (*AIRE*) gene, which encodes a transcription factor that induces the expression of peripheral tissue-specific antigens in thymic cells, regulates the negative selection of T-lymphocytes in the thymus, and plays an essential role in maintaining central immunological tolerance. The function of *AIRE* is not completely understood and continues to be studied [1, 2]. Mutations in *AIRE* are detected in the vast majority of patients with typical clinical manifestations of APS-1. Over 100 different mutations in *AIRE* have been described; however, each population has its own set of frequent mutations. In the Russian population, the most frequent mutation is the Finnish mutation R257, which forms a premature stop codon to produce a shortened protein [3]. Further, the presence of a wide range of organ-specific antibodies in patients with APS-1 confirms its autoimmune nature. Researchers have recently discovered a new class of anti-cytokine antibodies that are present in high concentrations in patients with APS-1. Some of these antibodies (anti-interferon-ω antibodies) are highly-specific and could be used as diagnostic markers [4].

APS-1 is characterised by three main clinical components observed in various combinations in 65%–100% of patients: mucocutaneous candidiasis, hypoparathyroidism and chronic primary adrenal insufficiency. The presence of at least two components is considered sufficient for a diagnosis. In addition to these major conditions, patients with APS-1 may develop minor symptoms, including alopecia, vitiligo, dental enamel hypoplasia, autoimmune hepatitis, autoimmune thyroiditis, pernicious anaemia, diabetes mellitus (DM), primary hypogonadism, hypopituitarism due to autoimmune hypophysitis and dry-eye syndrome [5, 6]; rare cases of retinopathy, autoimmune thrombocytopenia and red-cell aplasia have also been described [7, 8]. The severity of clinical manifestations varies from patient to patient, in the number of components (from 1 to 10), the age of onset and the time between the appearance of new components [5, 6, 9].

DM is a typical minor component of APS-1, with a prevalence of 2.5%–18% [3, 5, 10]. Clinical manifestations of DM in people with APS-1 are generally similar to those of type 1 DM (T1DM). The autoimmune nature of APS-1-associated DM was confirmed by the detection of anti-pancreatic antibodies [including antibodies to glutamic acid decarboxylase (GAD), antibodies to tyrosine phosphatase-related islet antigen 2 (IA2), pancreatic islet-cell antibodies (ICA) and insulin autoantibodies (IAA)] that are also considered to be T1DM markers [5, 11]. Zinc transporter 8 (ZnT8) antibodies have never been studied in patients with APS-1, and other investigators found no association between APS-1-associated DM and HLA class II haplotypes, which are believed to be predisposing to DM [11]. The monogenic nature of APS-1, known defects of cellular immunity and the insufficiency of other endocrine organs suggest that the onset and course of DM in these patients have specific characteristics.

AIM

To estimate the prevalence of DM among patients with APS-1 in Russia and to assess its clinical and immunological characteristics

METHODS

Study design

A total of 113 participants with APS-1 were enrolled; of these, 16 participants had DM. The participants were divided into two groups: group 1 included the 16 individuals with DM, and group 2 included 30 randomly selected individuals without DM.

We retrospectively assessed the clinical and laboratory data, including clinical characteristics, the presence of *AIRE* mutations, age at onset of DM, specific characteristics of DM onset, DM duration, insulin therapy, level of glycated haemoglobin (HbA1c), basal levels of glucose and insulin, level of anti-pancreatic antibodies and the results of a standard oral glucose tolerance test (OGTT) with estimated insulin levels.

In most of the patients, the *AIRE* gene was previously assessed by our research team [3].

An enzyme-linked immunosorbent assay was used to measure anti-ICA, anti-IA2, anti-IAA, anti-ZnT8 and anti-GAD antibodies at the Laboratory of Clinical Immunology and Genetics of the National Medical Research Center of Endocrinology, Ministry of Health of Russia.

Eligibility criteria

The diagnosis of APS-1 was based on the clinical criteria (presence of two of three main components) and/or detection of two mutations in the *AIRE* gene, as well as high serum levels of interferon-ω.

The diagnosis of DM was based on the criteria developed by the International Society for Pediatric and Adolescent Diabetes in 2014 [14]: DM symptoms in combination with occasionally detected plasma glucose ≥ 11.1 mmol/L; fasting plasma glucose ≥ 7.0 mmol/L; 2-h post-load glucose ≥ 11.1 mmol/L during an OGTT and HbA1c > 6.5%.

Study conditions

This study was conducted at the National Medical Research Center of Endocrinology, Ministry of Health of Russia.

Study duration

This was a retrospective study.

Description of medical intervention

All participants underwent blood collection from a peripheral vein for biochemical and hormonal analysis. Participants aged >18 years underwent a standard OGTT with 75 g of glucose; in paediatric participants, the glucose dose was calculated according to the total body weight (1.75 g/kg, but not >75 g). All participants who received any medical interventions within the study signed (either participants or their legal representatives) an informed consent form.

Primary outcome of the study

We described a unique group of patients with APS-1 and DM, estimated the prevalence of DM among individuals with APS-1 and assessed the sensitivity and specificity of laboratory testing for specific antibodies.

Secondary outcomes of the study

We described clinical cases of atypical DM.

Subgroup analysis

Study participants were divided into two groups: those with DM and those without DM. The presence or absence of DM was considered the main criterion.

Outcome measures

Outcome measures included clinical examination, biochemical and hormonal tests and molecular analysis of the *AIRE* gene.

Ethical review

The study protocol was approved by the Ethics Committee of the National Medical Research Center of Endocrinology (Protocol No. 11 from 23.10.2013).

Statistical analysis

Principles of sample size calculation. The sample size was not calculated.

Methods of data analysis. Statistical analysis was performed with the software package Statistica 8. We calculated means, medians, interquartile ranges, sensitivity and specificity with standard methods. We constructed contingency tables and used Pearson's $\chi 2$ test to estimate the difference between the groups. The Mann–Whitney U test was used to compare the means of the two independent samples.

RESULTS

The study included 113 participants with APS-1.

Main results of the study

A total of 113 participants with APS-1 with a median age of 19.4 (range: 2.7–44.6) years [female-to-male ratio: 1.26 (63/50)] were registered at the Institute of Pediatric Endocrinology by the time of study initiation. Sixteen participants (14.2%) had DM (Table 1).

Group 1. Participants with aps-1 and dm

Group 1 contained 16 participants with a median age of 19.4 (range: 13.0–25.5) years (female-to-male ratio of 1:1). The classical clinical triad of major components was observed in six participants. The median number of components was 5 (range: 2–10).

Table 1. Characteristics of APS-1 patients with and without DM

	Group 1	Group 2	Specificity	Sensitivity	p
Number of participants	16	30			
Median age	19.5	23			
Median number of components	5	5			
Presence of two or three major components, %	87.5	86.7			
Positive for at least one antibody type (anti-IAA, anti-IA2, anti-ICA or anti-GAD), %	100	33			<0.01
Positive for anti-ICA antibodies	0	3.3			>0.05
Positive for anti-IA2 antibodies	42	0	100	42	<0.01
Positive for anti-GAD antibodies	58	30	70	58	>0.05
Positive for anti-IAA antibodies	0	6.7			>0.05
Positive for anti-ZnT8 antibodies	33	3.3	97	33	<0.01

Note: IAA, insulin autoantibodies; ICA, islet-cell antibodies; IA2, islet antigen 2; GAD, glutamic acid decarboxylase; ZnT8, zinc transporter 8

Table 2. Manifestation of APS-1 components, mutation in the AIRE gene, autoantibodies in participants with APS-1 and DM

Partici-pant No.	Age	Components (age at onset)	Age at DM onset	*Mutations in the AIRE gene*	Insulin therapy	HbA1c	anti-ICA AB (N. <0,95)	anti-IA2 AB (N. <8)	anti-GAD AB (N. <1,0)	anti-IAA AB (N. <10)	anti- ZnT8 AB (N. <15)
1	27	CPAI (7), HPT (6), CMCC (5), AH (6), HT (11), M (6), PA (2+)	13	R257*/R257*	+	9.0	-	-	+	-	-
2	11	HPT (5), V (9), HT (9), DEH (9), M (9), CPAI (1+)	8	R257*/R257*	+	9.2	-	+	-		+
3	19	HPT (11), CPAI (13), V (17), TIN?	15	T16M/821delG	-	6.6	-	+	+	-	+
4	15	CPAI (12), PA (5), V (9), M (5), DEH (12)	13	p.Leu323serfs*51/ P326L	-	5.1	-	+	-	-	+
5	5*	CPAI (5), CMCC (1), M, AH (3), HT (5)	1.5	R257*/R257*	+	n/a					
6	22*	HPT (6), CMCC (11), CPAI (11)	2+	R257*/R257*	+	5.3					
7	26	HPT (1+), CMCC (1), AH (15), PA (1+), A (15)	9	R257*/R257*	+	6.8	-	-	+	-	-
8	11	CMCC (3), HPT (4), CPAI (5)	2.8	R257*/A58V	+	8.0	-	+	+	-	+
9	15*	CMCC (1), MD (4), HPT (7), M (6), A (6), CPAI (14), HT (7), PA (1+), CRF (13), DGR(7)	12	R257*/R257*	+	-	-	-	+	-	-
10	28	HPT (1), CPAI (14), V (13), RP, PT (14), HG (14)	14	R257*/R257*	+	9.6			+		
11	21	HPT (15), CPAI (15), CMCC (15), M, A(15)		R257*/R257*	+	7	-	-	+	-	-
12	28	CMCC (1+), HPT (11), M (4), V (19)		R257*/R257*	+	n/a					
13	23	HPT (10), CPAI (14), A, DEH, V (20) RP (2,5)		R257*/R257*		7.2	-	-	-	-	-
14	21	CMCC (0), AH (4), CPAI		R257*/R257*		n/a	-	-	+	-	-
15	11	HPT (3), CMCC (5), CPAI (5), DEH, M (8), A(8)		R257*/R257*		6	-	+	-	-	-
16	12	DM (9), HPT (10)	9	T16M/ K83E	+	10.5	-	-	+	n/a	-

Note: CPAI, chronic primary adrenal insufficiency; HPT, hypoparathyroidism; CMCC, chronic mucocutaneous candidiasis; AH, autoimmune hepatitis; HG, hypogonadism; HT, hypothyroidism; M, malabsorption, DEH, dental enamel hypoplasia; V, vitiligo; PA, pernicious anaemia; A, alopecia; MD, metaphyseal dysplasia; CRF, chronic renal failure; RP, retinitis pigmentosa; PT, ptosis; ND, no data, TIN, tubulointerstitial nephritis; DGR, duodeno-gastric reflux; AB, antibodies

+, high titre of antibodies

–, low titre of antibodies

Empty cell indicates that testing was not performed

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Table 3. Oral glucose tolerance test of participant No. 3

Parameters	0 min		30 min		60 min		90 min		120 min	
Time from DM diagnosis, months	0	24	0	24	0	24	0	4	0	24
C-peptide, ng/mL	2.41	1.3	3.5	1.1	4.4	1.9	6.3	2.3	8.2	4.7
Insulin, μU/mL	7.44	3.0	14.5		18.7	8.0	30		35	15.6
Glucose, mmol/L	5.9	4.7	8.1	6.8	15.1	11.7	16	14	14.6	16.5

Oral glucose tolerance test of participant No. 3

Table 4. Oral glucose tolerance test for participant No. 4

Parameters	0 min		30 min		60 min		90 min		120 min	
Time since DM diagnosis, months	0	8	0	8	0	8	0	8	0	8
C-peptide, ng/mL	1.8	1.1	3.1	1.8	4.4	2.9	5.1	2.0	6.2	3.8
Insulin, μU/mL	9.91	4.3	28	8.4	44.3	21.2	54.4	23.1	64.1	20.0
Glucose, mmol/L	3.9	4.3	5.5	4.8	6.7	7.7	8.0	8.7	8.4	8.5

Note: oral glucose tolerance test with 75 g of glucose monohydrate and with assessing postprandial levels of glucose, insulin and C-peptide after 30, 60, 90 and 120 min

In 13 participants, the diagnosis of DM was based on symptoms (polyuria, polydipsia, weight loss, glucosuria

and ketosis) and plasma glucose of ≥11.1 mmol/L. These patients received insulin therapy. Three patients presented no clinical symptoms of hyperglycaemia and were diagnosed with imp*AIRE*d glucose metabolism at OGTT (patient nos. 3, 4 and 16 in Tables 2, 3 and 4).

The median age at diagnosis of glucose metabolism disorder was 13 (range: 9–17) years. The median time between the onset of a first APS-1 component and DM diagnosis was 9 (range: 4–10) years, and the median duration of DM was 2.5 (range: 1.00–4.75) years. Thirteen out of the 16 patients had received insulin therapy by the time of study initiation. Patients 3 and 6 initially received 1–2 units of long-acting insulin; however, their insulin needs gradually decreased and therapy was ended. Currently, normoglycaemia is maintained by diet (without insulin therapy) in two patients (Nos. 4 and 16). In one patient (No. 3), DM was managed without insulin therapy for 4 years; he currently receives insulin only after high-carbohydrate meals (1–2 units of insulin per meal containing over 40 g of carbohydrates). In patients receiving insulin therapy, the median daily dose of insulin was 0.83 U/kg/day (range: 0.60–94), and the median level of HbA1c was 7.2% (range: 6.0–9.2) (Table 2).

Twelve participants with DM were tested for anti-GAD, anti-ICA, anti-IA2 and anti-ZnT8 antibodies; 11 participants were tested for anti-IAA antibodies.

High titres of autoantibodies to at least one antigen were detected in 11 participants. High titres of anti-IA2, anti-ZnT8 and anti-GAD antibodies were found in 42% (5/12), 33% (4/12) and 58% (7/12) of participants, respectively. None of the participants had both anti-ICA and anti-IAA antibodies.

Three participants from group 1 died at the age of 12, 15 and 33 years. In two patients (Nos. 5 and 6), the exact cause of death was unknown (they were not followed up within the centre during the last year); patient No. 9 died from chronic renal failure due to tubulointerstitial nephritis.

Mutational analysis of the *AIRE* gene demonstrated that 12 participants were homozygous for the R257* stop mutation; whereas, four participants harboured compound heterozygous mutations T16M/821delG, p.Leu323sefs*51/P326L, 257X/A58V, R257*/S185* and T16M/ K83E.

Clinical characteristics and genotypes of participants from group 1 are shown in Table 2.

Group 2. Participants with aps-1 but without dm

Group 2 was comprised 30 patients with a median age of 23 (range: 16.0–28.0) years (male-to-female ratio of 1:1.6). The median number of clinical APS-1 components was 5 (range: 2–10).

The R257* mutation in the *AIRE* gene was detected in 85% of alleles: 73.3% of participants were homozygous, whereas 23.3% were heterozygous. Almost 20% of participants had other mutations in addition to R257*. One participant did not undergo genetic testing (the diagnosis was based on clinical manifestations and a high level of anti-interferon-ω). Another patient harboured a heterozygous R257* mutation, the second mutation could not be detected.

All patients in group 2 underwent immunological testing. Anti-GAD antibodies were found in 30% of participants, anti-ICA antibodies in 3.3%, anti-IAA antibodies in 6.7%, anti-IA2 antibodies in 0% and anti-ZnT8 antibodies in 3.3% of participants (Table 1).

We have provided a detailed description of three participants with APS-1 and specific disorders of glucose metabolism.

Participanto. 3

At the time of our study, participant No. 3 was 18 years old, born to non-consanguineous parents and diagnosed at 11 years old with hypoparathyroidism based on specific symptoms (convulsive syndrome, hypocalcaemia, hyperphosphatemia and low levels

of parathyroid hormone); he was followed up at the Institute of Pediatric Endocrinology since diagnosis. At 12 years old, he presented with weakness, skin darkening and primary adrenal insufficiency, which resulted in the diagnosis of APS-1 based on clinical manifestations. Compound heterozygous mutations (T16 [T, M] and 821delG) were detected in the *AIRE* gene.

At the age of 16 years, he was diagnosed with DM during a routine examination. His 2-h postprandial plasma glucose was 14.4 mmol/L at OGTT and his HbA1c was 6.4%. The levels of insulin and C-peptide were within normal limits; their maximal glucose-stimulated concentrations during OGTT were 29.3 μU/L and 5.2 ng/mL, respectively. The levels of anti-ICA and anti-IAA antibodies were not elevated, anti-GAD antibodies were slightly increased (1.01 U/mL; reference range: < 1 U/ml), and anti-IA2 antibodies were not measured. Continuous monitoring of blood glucose with a usual diet demonstrated the maximum plasma glucose of 10.5 mmol/L. The patient received glucocorticoids (hydrocortisone) in a substitutional dose of 9.5 mg/m2/day, which should not have produced a glucose metabolism disorder. The patient was then given insulin detemir at a dose of 4 U/day, which normalised his glucose level. After discharge, insulin was ceased, but the patient adhered strictly to a low-carbohydrate diet equivalent to 5–7 bread units (BU) per day.

One year after the diagnosis of DM, his HbA1c level was 5.8%. His 2-h postprandial plasma glucose level during OGTT reached 14.6 mmol/L; however, his insulin secretion rate remained within the normal range (Table 3). Glucose monitoring with a continuous glucose monitoring system showed a maximum glucose level of 9.7 mmol/L. Immunological testing demonstrated a slight increase in the titre of anti-GAD antibodies (1.12 U/L; reference range: 0–1 U/L) and anti-IA2 antibodies (19 U/L; reference range: 0–15 U/L). Anti-ZnT8 antibodies were not measured at that time.

This individual continued to adhere to a low-carbohydrate diet (6–8 BU per day) and did not receive further insulin therapy. Two years after the diagnosis of DM, his HbA1c level was 5.8%. An OGTT revealed a decrease in the basal and stimulated levels of immuno-reactive insulin and C-peptide compared with previous testing, although these parameters were still within the normal limits (Table 3). Titres of anti-GAD, anti-IA2, anti-ICA and anti-IAA antibodies were within the normal range; whereas, the level of anti-ZnT8 was increased (27 IU/mL; reference range: < 15 IU/mL).

The patient's body weight remained stable on the low-carbohydrate diet (BMI: 19.24 kg/m2).

Thus, this individual with classic APS-1 had normal levels of HbA1c, C-peptide and insulin during the 2 years after being diagnosed with DM, allowing him to avoid insulin therapy during this period.

Four years after the diagnosis of DM, his HbA1c level reached 6.6% and postprandial glucose reached 15 mmol/L. The patient was given small doses of insulin as part only after consuming high-carbohydrate meals (1–2 units of insulin per meal containing over 40 g of carbohydrates).

Participant No. 4

This participant was followed up at the Institute of Pediatric Endocrinology. At the age of 3 years, he experienced a sharp decrease in hearing after a viral infection and was diagnosed with sensorineural hearing loss. At 5 years old, he was diagnosed with vitamin B12 deficiency anaemia and developed symptoms of malabsorption. Four years later, he presented with vitiligo on the face. At 11 years old, he was diagnosed with primary adrenal insufficiency; this age of onset, pernicious anaemia and vitiligo are typical of primary adrenal insufficiency, which can be a component of APS-1. The boy had high levels of anti-interferon-ω antibodies, confirming APS-1. This individual also harboured two *AIRE* mutations: p.R257* and p.P326L. He received replacement therapy with Cortef (10.2 mg/m2/day), Cortineff and vitamin B12 and underwent regular examinations recommended for APS-1 patients (regular glucose and HbA1c measurements).

At 13 years old, he was found to have elevated HbA1c and an OGTT was recommended. His 2-h postprandial plasma glucose level was 8.4 mmol/L. Immunological testing demonstrated high levels of anti-IA2 (400 U/mL) and anti-ZnT8 (500 U/mL) antibodies along with low levels of anti-GAD, anti-IAA and anti-ICA antibodies. At the time of examination, the patient was obese (standardised BMI, BMI SDS +2.25), so a low-calorie, low-carbohydrate diet was recommended (Table 4). The patient adhered to this diet (up to 6 BU per day) and achieved significant weight loss (BMI SDS +1.72)

A follow-up OGTT showed imp*AIRE*d glucose tolerance, normal insulin secretion and decreased basal and stimulated levels of immuno-reactive insulin and C-peptide compared with the previous OGTT (Table 4).

Considering the presence of a genetic autoimmune disorder with a high likelihood of developing autoimmune DM, high titres of antibodies against IA2 and ZnT8 and decreased insulin secretion, we assumed that the patient was experiencing the onset of latent autoimmune DM.

Participant No. 6

At 20 years old, this individual with classic APS-1 (having three major components) was diagnosed with hyperglycaemia (18 mmol/L) without ketoacidosis and started to receive insulin at a dose of 10 U/day, which maintained normoglycaemia. One year after initiating the therapy, insulin was ceased due to severe hypoglycaemia. During the next 2 years, blood glucose remained within the normal limits without any glucose-lowering therapy and diet. Three years later, the patient manifested DM with an HbA1c level of 5.3%. At this time, the patient was lost to follow-up, and we have no data on his glucose levels. The patient died at the age of 33, presumably from acute adrenal crisis.

Additional results of the study

We described cases of atypical DM in patients with APS-1.

Adverse events

No adverse events were reported.

DISCUSSION

Summary of the main study results

We found that the prevalence of DM in Russian patients with APS-1 is 14.1%. Of these, 19% had slowly progressing atypical DM. We also calculated the diagnostic sensitivity and specificity of anti-GAD, anti-IA2, anti-ICA, anti-IAA and anti-ZnT8 antibodies for diagnosing DM.

Discussion of the main study results

The prevalence of DM among Russian patients with APS-1 reached 14.1%, which is quite high compared with other nationalities. In Sardinians, the rate of DM among APS-1 patients is 5.2%; in Iranian Jews, it is 4% and in the French population, it reaches 5% [5, 11, 12, 13]. Only one Finnish study, with the largest sample size and the longest follow-up period, demonstrated an 18% prevalence of DM among individuals with APS-1. In the Russian cohort, the median age of these patients (at the time of study initiation) was 19.4 years, whereas the Finnish patients were significantly older (median age: 32.9 years); therefore, the number of Russian patients with DM may increase over time.

Such fluctuations in the prevalence of DM among patients with APS-1 in different countries are probably associated with the varying incidence of DM in the general population. In some countries (Finland, Norway, Sweden and Canada), the incidence is very high; whereas, in other countries (China, Thailand, Korea and Japan), it remains low [14]. For example, the incidence of T1DM in Finland is 52.6 per 100,000, which correlates with a high prevalence of DM (18%) among patients with APS-1 [15]. Curiously, despite the high prevalence of T1DM in the general population of Norway, its prevalence among individuals with APS-1 remains low (9%). In Russia, the incidence of T1DM is relatively low (11.01 per 100,000), which does not explain the high prevalence of DM among patients with APS-1 observed in this study.

In both the Finnish and Russian population, the most frequent *AIRE* mutation is R257*; whereas, patients from Norway and Sardinia are more likely to have other mutations [3, 5, 9]. It is possible that this genotype is a predisposing factor for DM, but there is still no evidence for this. Moreover, not all patients from group 1 in this study had this mutation [3].

According to the literature, diabetic patients with APS-1 have the same autoantibodies as patients with T1DM alone (anti-GAD, anti-IA2 and anti-ICA) [11, 12]; anti-ZnT8 antibodies have not been previously studied in individuals with APS-1.

Several studies suggest that patients with T1DM have anti-GAD antibodies in 64%–75% of cases, anti-IA2 antibodies in 61%–77% of cases, anti-IAA antibodies in 44%–92% of cases, anti-ZnT8 antibodies in 61%–80% of cases and anti-ICA antibodies in 81%–84% of cases [16-19]. Antibodies against at least one of the first three antigens are detected in approximately 86% of patients with T1DM; antibodies to at least one of the four listed antigens are found in nearly 96% of cases [17, 18]. We should also mention that the presence of anti-pancreatic antibodies, such as anti-ZnT8, correlates with a shorter duration of DM [16], but we failed to find any studies evaluating the level of anti-ZnT8 antibodies in patients with APS-1. The levels of anti-GAD, anti-IA2, anti-IAA and anti-ICA in patients with APS-1 were estimated by many authors. Anti-GAD and anti-IAA antibodies were equally and frequently detected in patients with APS-1 and DM and in individuals with DM alone; whereas, anti-IA2 antibodies were detected more frequently in patients with T1DM than in patients with APS-1 and DM. In our study, the level of anti-IA2 antibodies was elevated in half of the patients.

There are several studies that estimated the level of anti-IAA, anti-IA2, anti-GAD and anti-ICA antibodies in patients with APS-1. The study by Gilling et al. included 68 Finnish participants with APS-1; of these, 12 had DM. Eleven patients were tested for autoantibodies prior to clinical manifestations and at the time of DM onset; one patient was tested only 2 years after disease manifestation. Among these 11 patients, anti-IA2 antibodies were identified in 36.3% of cases, anti-IAA antibodies in 36.3%, anti-ICA antibodies in 54.5% and anti-GAD antibodies in 72.7% of cases. The last patient was positive for anti-GAD but negative for the other antibodies, which can be explained by late testing. In 48 non-diabetic patients, anti-IA2, anti-IAA, anti-ICA and anti-GAD antibodies were found in 4%, 0%, 54.5% and 22.9% patients, respectively. These findings suggest that anti-IA2 and anti-IAA are more specific (specificity was 95.8% and 100%, respectively) but less sensitive (sensitivity of both was 36.3%) than anti-GAD (specificity, 64.6% and sensitivity, 72.2%) and anti-ICA antibodies (specificity, 79.2% and sensitivity, 54.5%) [11]. The low specificity of anti-GAD antibodies can be attributed to the fact that this antigen is also expressed in the nervous tissue; therefore, anti-GAD antibodies should not be considered as reliable markers of autoimmune diabetes in patients with APS-1.

The study by Proust-Lemoine et al. included 14 French patients with APS-1; only one was diagnosed with DM. Among these 14 patients, anti-GAD antibodies were detected in five patients (36%), anti-IA2 antibodies in one (7%) and anti-ICA antibodies in two (14%). The patient with DM had anti-GAD and anti-IA2 antibodies, but no anti-ICA antibodies [13].

Our results suggest that anti-ZnT8 and anti-IA2 antibodies are highly specific (97% and 100%, respectively) in patients with DM and APS-1; however, their sensitivity is low (33.3% and 42%, respectively). Anti-GAD antibodies are more sensitive, but less specific.

ZnT8 is an intra-cellular transporter involved in zinc homeostasis and ensures the structural stability of insulin [20]. Antibodies against ZnT8 were not previously measured in patients with APS-1. In our study, anti-ZnT8 antibodies were found to be highly specific in diabetic patients with APS-1, although their sensitivity was low. Thus, a positive result for anti-ZnT8 antibodies indicates a high probability of DM; whereas, the absence of anti-ZnT8 is not a reliable prognostic marker.

APS-1 is characterised by polymorphic symptoms that may vary even within the same family. DM may develop in one sibling but not in the second one, despite the identical genotype [21]. Thus, there are likely some additional factors affecting the manifestation of DM that have not been identified. The study by Paquette et al. included 50 patients with APS-1; of them; eight individuals had DM. They genotyped the 5'INS VNTR IDDM2 locus and found a positive correlation between DM and shorter 5'INS VNTR class I alleles and vice versa (a negative correlation with longer 5'INS VNTR class III alleles), but this correlation was significant only in patients with two 5'INS VNTR class I alleles. Among diabetic patients, class I/I was observed in 87.5% of patients and class I/III and III/III in 12.5% of patients. Among non-diabetic patients, class I/I was found in 59.4% of patients and class I/III and III/III in 40.5% of patients. The authors suggested that the 5'INS VNTR class I allele may play a significant role in predisposing people with APS-1 to DM [21].

Some researchers looked for an association between DM in people with APS-1 and HLA class II DQA1, DQB1 and DRB1 polymorphisms. A negative correlation was identified between the protective allele DQB1*0602 and DM in individuals with APS-1; the prevalence of this allele was similar to its prevalence in the general Finnish population [11]. Nevertheless, the researchers failed to find any correlation between the predisposing haplotypes and DM or between protective or predisposing haplotypes and the level of anti-GAD antibodies [11]. We did not perform HLA-typing for our participants.

It appears quite difficult to compare the levels of HbA1c between diabetic and non-diabetic patients with APS-1 due to the small sample size of patients with APS-1 and the close association of this parameter with the quality of self-blood glucose monitoring.

We paid particular attention to participants with slowly progressing DM. Participant No. 3 is quite interesting due to his mild DM and prolonged complete remission with normal insulin secretion (both insulin and C-peptide levels were within the normal range), despite the high titres of anti-GAD and anti-IA2 antibodies.

Complete remission of T1DM in children is a rare event, occurring in only 1%–3% of patients; whereas, partial remission is observed in 68% of children. The duration of this remission is usually 7.2 ± 4.8 months [22]. One of our patients experienced remission for 4 years, although follow-up examinations demonstrated a gradual decrease of insulin and C-peptide secretion and increasing levels of HbA1c.

The gradual development of DM, observed in patients with APS-1, is probably associated with regular monitoring of blood glucose and early diagnosis. We should not ignore specific characteristics of autoimmune processes in individuals with defects in the *AIRE* gene. The contradictory results of immunological testing in participant No. 3 (high levels of autoantibodies and their disappearance 1 year later along with preserved insulin and C-peptide secretion) can be attributed to a 'wave-like' autoimmune process. Imp*AIRE*d glucose tolerance in participant No. 4, together with high levels of anti-IA2 antibodies (highly-specific for autoimmune DM), may indicate the similarity between his DM and latent autoimmune DM in adults, characterised by the secretion of specific antibodies and slow development of insulin dependence (during 3–6 years) [23].

It is possible that the autoimmune process is initiated by other antibodies that have not yet been identified, so the production of anti-IA2, anti-ICA and anti-GAD is a result of ongoing destruction in the endocrine pancreas. High titres of these antibodies in non-diabetic patients with APS-1 reduce their specificity and prognostic value, which requires further study. The assessment of new antibodies against ZnT8, cytokines and interferons in larger samples, as well as the evaluation of other genetic factors (for example, genotyping of the 5'INS VNTR IDDM2 loci), will provide valuable information about new pathogenetic mechanisms underlying APS-1.

Limitations of the study

Some of the patients were not tested for antibodies due to various reasons (lack of blood samples or informed consent).

CONCLUSION

The prevalence of DM in Russian people with APS-1 reaches 14.1%. Of these, 19% have slowly progressing, atypical DM with a long period of preserved insulin secretion and normoglycaemia without insulin therapy. Thus, the regular assessment of glucose metabolism in patients with APS-1 is crucial for a timely diagnosis of DM.

Further studies with larger sample sizes are needed to estimate the diagnostic value of anti-pancreatic antibodies for DM in patients with APS-1. Nonetheless, it is already clear that patients with high serum levels of these antibodies require careful monitoring due to the high risk of developing glucose metabolism disorders.

Our findings suggest that anti-GAD antibodies are less specific and can be detected in both diabetic (58.3%) and non-diabetic (30.0%) patients because this antigen is expressed in other organs and tissues. Conversely, anti-IA2 antibodies are highly specific; they were identified in 42% of our participants with DM and in none of the participants without DM. Finally, we are the first to assess anti-ZnT8 antibodies in patients with APS-1 and found that they are highly specific (97%) for DM. However, their sensitivity was

significantly lower (33.3%), restricting their use as a prognostic marker for DM in patients with APS-1.

ADDITIONAL INFORMATION

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Author contributions. Orlova E.M. developed a research concept and study design; Sozaeva L.S. and Orlocva E.M. performed data collection, processing and analysis and drafted the manuscript; Savelyeva L.V., Andrianova E.A, Svetlova G.N., Zilberman L.I. performed data collection and processing; Ivanova O.N. and Stepanova S.M. performed laboratory testing and edited the manuscript; Orlova E.M., Kareva M.A., Mayorov A.Yu., Troshina E.A., Khusbi E.S. and Peterkova V.A. edited the manuscript.

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