

Humoral response markers in GCK MODY

Markery reakcji humoralnej w cukrzycy GCK MODY

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Abstract

Background. The prevalence of antibodies to pancreatic islets in monogenic diabetes remains unknown and the incidence estimation is difficult as the occurrence of autoantibodies in patient is one of the well-known exclusion criteria for further genetic diagnostics. They has been found not only among patients with type 1 diabetes, but also in other types of diabetes: Type 2 diabetes, Latent Autoimmune Diabetes in Adults (LADA) (16) and monogenic diabetes (MD). **Aim.** Immunological characteristic of GCK MODY patients. **Methods.** The study group included families of 27 adolescent patients with GCK MODY (39 parents and 19 siblings) monitored in the Department of Pediatrics, Endocrinology and Diabetes and in the Diabetes Clinic of John Paul II Upper Silesian Child Health Centre in Katowice in the years 2007-2012. All patients and family members with GCK MODY underwent a blood sample drawing for immunological (classic humoral response markers: ICA, GAD, IA-2, IAA) and biochemical diagnostics. Pediatric, diabetes and family medical history was collected from the subjects and parents. **Results.** Immunological diagnostics was performed in all patients except 1 (96.3%). Immunological diagnostics included 17 (89.5%) parents and 7 (87.5%) siblings with diagnosed GCK MODY. 8 (30.8%) adolescent patients with GCK MODY, 3 subjects (17.64%) among parents (with GCK MODY), as well as 2 subjects (28.57%) among siblings (with GCK MODY) showed a positive antibodies screen. **Conclusion.** The results of our study in children with GCK MODY and their family members suggest that the occurrence of classic antibodies directed against pancreatic islets antigens is fairly common in patients with GCK MODY. Despite various observations and many legitimate discussions, it is difficult to clarify the pathogenesis of the occurrence of autoantibodies in monogenic diabetes.

Key words:

GCK MODY, autoantibodies, monogenic diabetes, GAD, ICA, IAA, IA2

Streszczenie

Wstęp. Rozpowszechnienie występowania przeciwciał przeciwko antygenom wysp trzustkowych w cukrzycy monogenowej nie jest znane, a oszacowanie częstości występowania jest trudne, ponieważ jednym z powszechnie znanych kryteriów wykluczających pacjenta z dalszej diagnostyki genetycznej jest obecność autoprzeciwciał. Obecność przeciwciał przeciwko antygenom wysp trzustkowych stwierdzono nie tylko wśród pacjentów z typem 1, ale również w innych typach cukrzycy: cukrzycy typu 2 i LADA (Latent Autoimmune Diabetes in Adults, późno ujawniająca się cukrzyca o podłożu autoimmunologicznym u osób dorosłych) oraz w cukrzycach monogenowych (MD, Monogenic diabetes). **Cel.** Charakterystyka immunologiczna pacjentów z cukrzycą GCK MODY. **Materiał i metodyka.** Grupa badana obejmowała rodziny 27 młodocianych pacjentów z cukrzycą GCK MODY (39 rodziców oraz 19 rodzeństwa) pozostających pod opieką diabetologiczną w Oddziale Pediatrii, Endokrynologii i Diabetologii Dziecięcej z Pododdziałem Diabetologii Dziecięcej oraz Poradni Diabetologicznej GCZD w Katowicach w latach 2007–2012. U wszystkich pacjentów i członków ich rodzin z GCK MODY, po wyrażeniu przez nich i ich rodziców/opiekunów pisemnej zgody na udział w badaniu, pobrano próbkę krwi celem przeprowadzenia diagnostyki immunologicznej (klasyczne markery reakcji humoralnej: ICA, GAD, IA-2, IAA) oraz biochemicznej. Zebrano wywiad ogólnopediatryczny, diabetologiczny oraz rodzinny. **Wyniki.** Diagnostykę immunologiczną

wykonano u wszystkich pacjentów z wyjątkiem jednego (96,3%). Diagnostyką immunologiczną objęto 17 (89,5%) rodziców oraz 7 (87,5%) rodzeństwa z rozpoznaną cukrzycą GCK MODY. Dodatkowo przeciwciała odnotowano u 8 (30,8%) młodocianych pacjentów GCK MODY, wśród 3 (17,64%) rodziców (z cukrzycą GCK MODY) oraz wśród 2 (28,57%) rodzeństwa (z cukrzycą GCK MODY).

Wnioski. Wyniki badania dzieci z cukrzycą GCK MODY i członków ich rodzin dowodzą, że występowanie klasycznych przeciwciał skierowanych przeciw antygenom wysp trzustkowych jest dość częstym zjawiskiem wśród pacjentów z cukrzycą GCK MODY. Mimo różnorodnych obserwacji i wielu zasadnych rozważań trudno jednoznacznie wyjaśnić patogenezę pojawienia się autoprzeciwciał w cukrzycy monogenowej.

Słowa kluczowe:

GCK MODY, przeciwciała przeciwko komórkom β trzustki, cukrzyca monogenowa, GAD, ICA, IAA, IA2

Introduction

The prevalence of antibodies to pancreatic islets in the monogenic diabetes remains unknown and the incidence estimation is difficult as the occurrence of autoantibodies in the patient is one of the well-known exclusion criteria for further genetic diagnostics [1–3]. This approach can lead to an underestimation and unreliably respond to the matter of the humoral response markers in diabetes of non-autoimmune etiology. Antibodies directed against pancreatic islets antigens are thought as detectable indicator of the ongoing process of pancreatic islets β cells destruction [4]. They are a valuable predictive marker for the development of type 1 diabetes [5]. They has been found not only among patients with type 1 diabetes, but also in other types of diabetes: Type 2 diabetes and monogenic diabetes (MD) [6–10].

Aim of the study

The presence of β -cell autoantibodies in GCK MODY patients.

Materials and Methods

The study group included families of 27 adolescent patients with GCK MODY (22 families; 39 parents: 22 mothers, 17 fathers and 19 siblings) monitored in the Department of Pediatrics, Endocrinology and Diabetes and in the Diabetes Clinic of John Paul II Upper Silesian Child Health Centre in Katowice in the years 2007–2012.

All patients, their parents/legal guardians and family members with GCK MODY gave informed written consent to participate in the study. Subjects underwent a blood sample drawing for immunological and biochemical diagnostics. Pediatric, diabetes and family anamnesis was collected from the subjects.

The conventional autoantibodies were measured on serum samples: ICA with immunofluorescence, antibodies GADA, IA2A and insulin antibodies (IA/IAA) with RIA (CisBioInternational, France and RSR, USA). The cut-off values for ICA, GADA, IA2A and IA/IAA positivity were 10 Juvenile Diabetes Foundation units, 10U/ml and 20U/ml, 15U/ml and 7% or 0,4U/ml respectively. According to the Islet Autoantibody Standardization Program - IASP2015 the disease sensitivity of the antibody

was ICA: 72.3%, GADA: 82%, IA2A: 70% and IA/IAA 42% respectively, while corresponding specificities were; ICA: 94,4%, GADA: 98.9%, IA2A: 95.6%, IAA: 100%. Genetic diagnostics (HLA genes – by PCR-SSP system using sets of INNO-LIPA Innogenetics, Belgium) and immunological diagnostics was performed at the Laboratory of Immunopathology and Genetics in the Department of Pediatrics of the Medical University of Lodz.

Biochemical Diagnostics

1. Glycated hemoglobin test HbA_{1c} (by high-performance liquid chromatography) in laboratory of The Independent Public Clinical Hospital no. 1 of the Medical University of Silesia in Zabrze and in the Silesian Analytical Laboratories.

2. The oral glucose tolerance test (OGTT) according to the WHO, performed in patients not meeting the diabetes criteria in casual plasma glucose sample. This test involves administering of 1.75 g of glucose per kilogram of body weight, but a maximum of 75 g of glucose to a patient, and plasma glucose measurements at two time points (before the administration of the glucose and after 2 hours).

3. The C-peptide concentration (using the electro-chemiluminescence immunoassay (ECLIA) on Roche elecsys module immunoassay analyzer) in the laboratory of The Independent Public Clinical Hospital no. 6 of the Medical University of Silesia in Katowice.

4. Biochemical tests: total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), ALT, AST, creatinine, urea, pituitary hormone (TSH) and thyroid hormone (free thyroxin fT4) concentration (by chemiluminescent immunometric assay), anti-thyroid antibodies: thyroglobulin antibodies (ATG), anti-thyroid peroxidase (TPO) (by chemiluminescence).

The patients were divided into two subgroups, depending on the immune profile. A group of patients with positive or negative antibodies were compared in terms of the age of diagnosis of glucose metabolism disturbances, type of the glucose metabolism disturbances, C-peptide levels and the HLA genes.

Results

The home institution, in line with the Polish Diabetes Association guidelines [11], in newly diagnosed diabetes in chil-

dren, routinely identifies 4 immunological markers to confirm or verify the diagnosis of type 1 diabetes. In the case of few patients, immunological diagnostics were done before the mentioned guidelines, which is why they have not determined all 4 basic types of antibodies.

A complete immunological diagnostics in adolescent patients, at diagnosis, defined as a determination of 4 basic types of antibodies – GAD, ICA, IAA, IA2, was performed in 20 patients (74.1%), 3 out of 4 above-mentioned types of immunological markers were determined in 4 patients (14.8%), 2 out of 4 in 2 patients (7.4%). Immunological diagnostics did not include one patient (3.7%). The detailed characteristic of the immune profile is presented in Fig. 1.

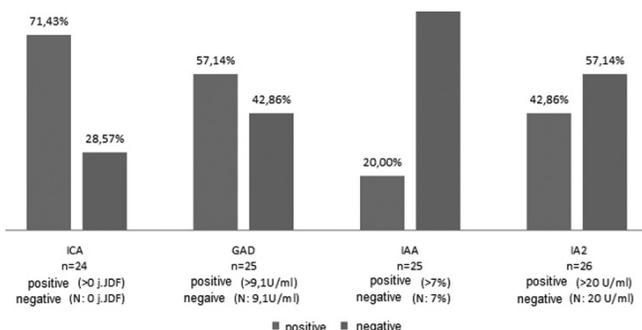


Fig. 1. The immune profile of children with GCK MODY diabetes
Ryc. 1. Profil immunologiczny dzieci z cukrzycą GCK MODY

Immunological diagnostics, with determination of 3 or 4 basic types of antibodies - GAD, ICA, IAA, IA2, included 17 (89.47%) parents and 7 (87.50%) siblings with diagnosed GCK MODY. Among them, 11 (57.89%) parents and 5 (62.5%) siblings underwent a complete immunological diagnostics, defined as a determination of 4 out of 4 above-mentioned types of antibodies. 3 out of 4 types of immunological markers were determined in 6 (31.58%) parents and 2 (25.0%) children. 3 subjects (60.0%) among parents and asymptomatic mutation carriers, as well as 2 subjects (15.38%) among siblings showed positive antibodies screen. The detailed characteristic of the immune profile is presented in Fig. 2–3.

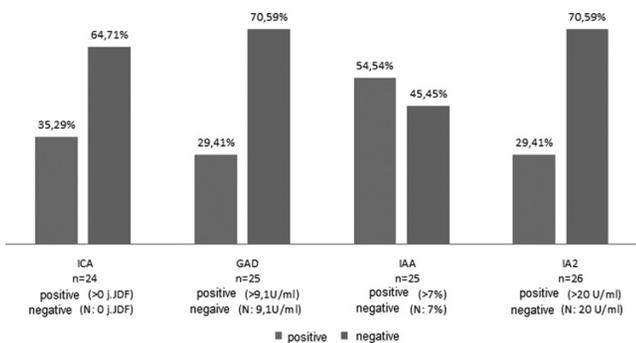


Fig. 2. The immune profile of parents with GCK MODY
Ryc. 2. Profil immunologiczny rodziców z GCK MODY

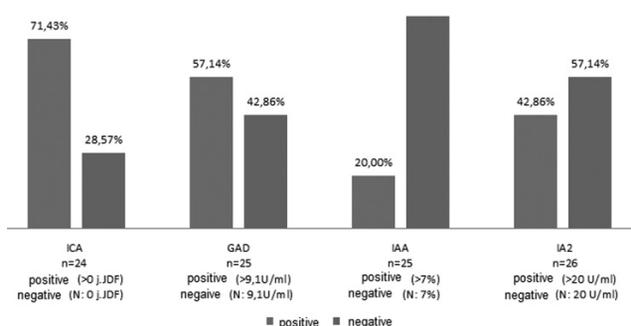


Fig. 3. The immune profile of siblings with GCK MODY
Ryc. 3. Profil immunologiczny rodzeństwa z cukrzycą GCK MODY

The mean age of onset of glucose metabolism disturbances of recruited patients was: 9.46 +/- 4.59 (years).

10 patients met the diagnostic criteria for diabetes in OGTT, 17 patients were diagnosed with IFG and/or IGT.

At the time of onset of glucose metabolism disturbances, 15 patients had a normal level of C-peptide (>0,5ng/ml), the lack of C-peptide was diagnosed in 8 patients (≤ 0.5 ng/ml).

The mean HbA1c at the age of onset of glucose metabolism disturbances were: 6.85 +/- 1.39 (%) (min. 5.5%, max. 12.22%), the mean HbA1c: 6.52 +/- 0.41 (%) (min. 5.61%, max. 7.67%).

8 parents (50,0%) met the diagnostic criteria for diabetes in OGTT, 7 parents (46,7%) were diagnosed with IFG and/or IGT. 1 parent (6.7%) had normal glucose levels in OGTT. The mean HbA1c: 6.34 +/-0.44 (%) (min. 5.40, max. 7.0).

2 siblings (33.3%) met the diagnostic criteria for diabetes in OGTT, 2 siblings (33,3%) were diagnosed with IFG and/or IGT. 2 siblings (33.3%) had normal glucose levels in OGTT. The mean HbA1c: 6.36 +/-0.85 (%) (min. 5.0, max. 7.7).

The statistical analysis has revealed no significant differences in the age of onset, the metabolic control nor the pancreas endocrine function between the subgroup with positive antibodies to pancreatic islets antigens and the subgroup with the negative expression of antibodies.

HLA genes haplotypes recognized as predisposing to diabetes type 1 were observed rarely (DQ8 – 3 subjects (11%), DQ2 5 subjects (18%)) with frequency typical rather for the general population than for diabetic patients [12]. There has been no correlation between the occurrence of specific HLA genes haplotypes and the occurrence of antibodies directed against antigens of pancreatic islets (Fig. 4).

An important limitation of our study is a small study group that, in addition, is very heterogenous. That is why our results have to be considered as observations which should be verified in the multicenter study including bigger study group.

Discussion

The autoantibodies in patients with monogenic diabetes are the topic of growing interest in modern diabetology. The lit-

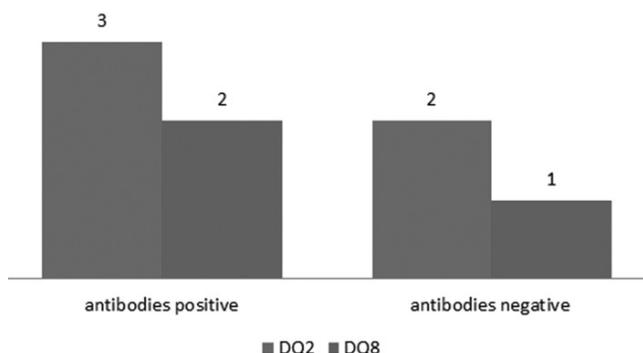


Fig. 4. The occurrence of specific HLA genes haplotypes
Ryc. 4. Występowanie specyficznych haplotypów genów HLA

erature review on one hand denies the presence of antibodies in patients with monogenic diabetes (13–15), on the other hand several studies confirm the presence of humoral response markers in patients with MODY [8–10,16,17]. In addition, there has been shown that the incidence of positive autoantibodies titer in patients MODY is 1–2%, which is comparable to its prevalence in the general population [18].

The results of our study in children with GCK MODY and their family members suggest that the occurrence of classic antibodies directed against beta cells antigens is fairly common in patients with GCK MODY. They are consistent with the preliminary nationwide data analysis involving 375 patients with GCK MODY [19], which suggest that the beta cells antigens (ICA, GAD, anti-IA2, IA / IAA and ZnT8.) are present in 23.2% (87/375) of patients with GCK MODY.

In terms of the above-mentioned reports, the presence of beta cells antigens may denote the coexistence of autoimmune diabetes and monogenic diabetes [8,9], or may reflect a late defect of pancreatic β cells as their destruction manifested by the presence of autoantibodies [17] or, lastly, their presence, as suggested by other authors [18], may be a mere coincidence having no pathophysiological significance.

An independent coexistence of monogenic diabetes and type 1 diabetes was first described by Maltoni et al. [9]. The authors presented a case of a child with GCK MODY at diagnosis with good metabolic control (HbA1c 6.6%), with negative expression of autoantibodies (GAD, ICA), that did not require any pharmacological intervention, with the typical GCK MODY mild disease. After a few months, an unexpected HbA1c deterioration required initiating insulinotherapy. They decided to re-determine the immune status of the patient, which revealed a seroconversion. According to the authors, an increase in blood glucose reflects the development of antibodies associated with the destruction of the pancreatic β cells. Their presence should be associated with the disclosure of diabetes type 1. Bowden et al. drew similar conclusions [8], finding out that more severe clinical course of MODY 3 in described patient was linked to the presence of antibodies, and thus the outbreak of type 1 diabetes. However, there was a patient in our study who presen-

ted severe ketoacidosis at the onset of diabetes. The glucose profile permitted the diagnosis of diabetes, and the positive autoantibodies expression confirmed the autoimmune etiology. Due to the different clinical course of diabetes in the patient's sister, which did not fit in with the course of T1DM, the molecular diagnostics was performed. It confirmed GCK MODY in the patient's sister. The patient was then involved in the molecular diagnostics, which found the same mutation in the GCK gene. The different course of diabetes in the described case suggests that the more severe clinical course in the brother is associated with the presence of the β cells destruction markers, typical of type 1 diabetes.

In order to establish the possible coexistence of the two types of diabetes, a HLA testing was performed in patients with the confirmed GCK MODY mutation. Theoretically, one would expect a positive correlation between the presence of antibodies and the haplotypes DQ8 and DQ2, which predispose to developing type 1 diabetes [12]. In our study, the correlation did not occur, but the relatively small study group may be insufficient to clearly assess this aspect.

However, the presence of autoantibodies is not always associated with more severe GCK MODY course. Among patients with a positive immunological profile in our study group, there have been 3 children, who do not require any pharmacological treatment. Similar observations can be found in the study of Ortega-Rodriguez et al. [20]. They described a patient with GCK MODY and positive expression of antibodies to antigens of pancreatic islets with a mild clinical course of diabetes, where there was no need for pharmacological support.

To evaluate the impact of the occurrence of autoantibodies on the clinical course of diabetes in patients with monogenic diabetes, the study group was divided into 2 subgroups, based on the presence of autoantibodies. The two groups were compared in terms of the age of onset, the metabolic control, the type of therapy, the endocrine function of the pancreas, which did not give any statistically significant differences. It can therefore be emphasized that the presence of the β cell destruction markers does not decide on the clinical course of GCK MODY in as conclusive way as one would expect, and each patient should be analyzed individually.

For proper functioning of the human body cells must undergo apoptosis. This is a physiological phenomenon occurring through the cell metabolism. The signal for apoptosis can be triggered in every cell of our body. It also concerns β cells of the pancreas. Among the various factors that can induce apoptosis in β cells there are metabolic pathways defects [17,21,22]. A defect in the glucokinase gene results in a dysfunction of the glycolysis pathway and perhaps increases the cellular turnover. A prolonged exposure of the immune system to the tissue-specific autoantigens, released through the apoptosis may ultimately lead to the induction of an autoimmune response. Therefore, it can be concluded that the antibodies to the antigens of pancreatic islets occur secondary to the β cell dysfunction. The time is probably an important factor. It has been described that the antibodies in patients with MODY [8,9] occurred over the diagnostic and therapeutic process. For patients included in this analysis it cannot be told whether, and

what is the dynamics of change of autoantibodies, as the immunological profile has been determined only once at the disclosure of the glucose metabolism defect, and there were no indications to verify the immune status of the patient later on.

The presence of autoantibodies in MODY still poses a number of questions, as it is a problem the solution to which solution is still actively searched for. The antibodies reflect the activation of the immune system, but they are not directly involved in the cellular destruction process [5,23]. The reciprocal interaction between the lymphocytes T-helper1 and T-helper2 (Th1 / Th2) underlies the pathogenesis of type 1 diabetes. A hypothesis under current consideration suggests that the autoimmune process is initiated by the non-pathogenic Th2 subpopulation, which may turn later on into the destructive process mediated by Th1 subpopulation, inevitably leading to the onset of type 1 diabetes [5,23]. Depending on the balance between lymphocyte Th1 / Th2-mediated processes, some patients may stay in the latent stage of diabetes, in the so-called "non-progressors"

group of patients who despite the presence of the immunological markers typical of type 1 diabetes, have no clinical manifestation of the disease. The hypothesis, very generally characterized, may give rise to attempt a more detailed research on why patients with a positive immune profile and monogenic diabetes should be monitored for type 1 diabetes.

Despite various observations and many legitimate discussions, it is difficult to clarify the pathogenesis of the occurrence of autoantibodies in the monogenic diabetes and to define which of the hypotheses is the most likely.

Acknowledgment

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