Autoimmunity Markers in Patients with Type 2 Diabetes

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ABSTRACT

Introduction

Diabetes is a growing social and epidemiological problem. Frequent diabetes complications occurrence has an impact on a higher percentage prevalence of the cardiovascular, kidneys, nervous system and vision diseases.

Aim

The aim of the study was to evaluate the incidence of immunological markers in patients with type 2 diabetes: the anti-GAD, ANA, AMA, ASMA, APCA, and LKM compared to healthy subjects. Another objective of the study was to evaluate the correlation between their presence and the degree of metabolic control in both groups.

Material and Methods

The study included 50 subjects aged 40-75 years with a Body Mass Index (BMI) between 20-30 kg/m^2 with previously diagnosed type 2 diabetes. The control group consisted of 21 healthy individuals without a diagnosis of neither diabetes nor a prediabetic state. All the study participants had the examined antibodies determined along with the panel of biochemical tests and neurological examination for diabetic neuropathy and fundus examination.

Results

Anti-GAD antibodies were present in 16% of patients with type 2 diabetes. The presence of ANA antibodies was found in 22% subjects. There was no correlation between the presence of ANA antibodies and diabetic microvascular complications. ASMA and APCA antibodies occurred with equal frequency in studied groups (4% vs. 10%). There were no antibodies of AMA or anti-LKM in any of the patients.

Conclusion

The presence of studied autoantibodies in patients with type 2 diabetes is frequent but cannot be a marker used to isolate a group of patients at risk of developing diabetic micro vascular complications. The presence of anti-GAD in type 2 diabetes may be a LADA marker which specifically marks a group of patients with type 2 diabetes, in who there is a faster metabolic death of beta cells. Determination of antibodies AMA, ASMA, APCA and anti-LKM does not seem to be significant in the diagnosis of diabetes and its chronic complications.

Abbreviations

ICA: Islet Cell Antibodies; IAA: Insulin Autoantibodies; AAEI: Antibodies against Endogenous Insulin; Anti-GAD: Anti-Glutamic Acid Decarboxylase Antibodies; Anti-IA-2: Antibodies against Tyrosine Phosphatases; ANCA: Anti-Neutrophil Cytoplasmic Antibodies; ANA: Antinuclear Antibodies; AMA: Anti-Mitochondrial Antibodies; ASMA: Anti-Smooth Muscle Antibodies; APCA: Antiparietal Cell Antibodies; LKM: Liver Kidney Microsomes Antibodies; BMI: Body Mass Index; WHR: Waist-Hip Ratio; HbA1C: Glycated

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Hemoglobin; EGFR: Estimated Glomerular Filtration Rate; ss:DNA: Single Stranded DNA Antibodies; HOMA: Insulin Resistance; SS: Sjogren's Disease

Introduction

Diabetes is not a single disease entity. Conducted over the past decades, research and clinical enabled the understanding of this heterogeneity. In 1997, it announced the etiological division of diabetes, which distinguishes two main forms: type 1 and type 2. In the pathogenesis of both forms genetic and environmental factors play the role. Type 1 develops as a result of autoimmune destruction of pancreatic islet B cells. This represents approximately 10% of all cases of the disease. Type 1 diabetes is associated with the presence of autoantibodies against antigens of B cells and carriers of certain alleles of the major histocompatibility passed to it more often than the general population [1,2].

Type 2 is the more common form, representing about 90% of all cases of the disease in the developed regions of the world civilization. It is characterized by the coexistence of insulin deficiency and the peripheral effects of the hormone [3,4]. Immunological markers do not appear, and the genetic basis has no connection with the HLA system.

In addition, there is rising incidence of type 2 diabetes in children and adolescents [5]. In the end, the last two decades have produced important findings about monogenic forms of diabetes, revealing mainly in the developmental age and young adults [6]. These findings have proved that diabetes has much more diverse etiology, than recently thought.

In the last few years, it has been shown that autoimmune diabetes in adults occurs much more often than it was previously thought [6].

Chronic autoimmune process leading to the destruction of the B cells of pancreatic islets is a cellular response and observed in type 1 diabetes antibodies associated with humoral response is a marker of ongoing autoimmune destruction process. The presence of antibodies can define a person at risk of developing type 1 diabetes as early as during the pre-clinical stage of disease [2]. The prevalence of anti-GAD antibodies in type 1 diabetes was found in 80-90% patients with newly diagnosed diabetes and 70 -80% in patients with the preclinical diabetes [7-9] is present preceded by several years of before clinical manifestation of the disease [10,11].

Circulating auto-antibodies in sera of type 1 diabetes patients have been noted to react with autonomic tissues, most notably symphatetic ganglia and the vagus nerve, which might be associated with future development of autonomic neuropathy [12]. Winer et al. stated that the pancreatic islets of Langerhans are surrounded by a Schwan cell sheath. There may be a direct destruction of neurons by the same autoimmune process in diabetes [13]. Autoimmune processes are also prevalent in population, showing that clinical autoimmune disorders can have a frequency up to 7.6-9.4% [14].

Immune dysregulation can be simple bystander or can be additional factor in destructive inflammatory processes. Diabetes type 2 with anti-GAD antibodies can be classified as diabetes type 1 or distinct form of diabetes type 2 with ad on autoimmune process connected with inherent in diabetes type 2 excessive death of beta cells.

The presence of anty-GAD in patient with recent onset type 1 diabetes is associated with hyperglycaemia and reduced peripheral nerve function, suggesting a common mechanism for β- cell and neuronal damage.

Patients with high anty-GAD antibodies were shown to have reduced motor nerve conduction velocities in the median, ulnar and peroneal nerves, prolonged F wave latencies, high thermal threshold detection for hot and cold, and decreased cardiovascular autonomic function [15].

The same can be connected with other other autoimmune processes autoantibodies. Increased destruction of cells in different organs due to microangiopathy and macroangiopathy can induce secondary immune response, which may aggravate further course of chronic complications. ANA antibodies which are produced in response to cell necrosis or cell apoptosis can be marker or can be directly involved in chronic complications of diabetes. As an example of this association could be notion made on higher prevalence of antinuclear antibodies in more severe forms of coronary atherosclerosis [16]. Serum from patients with diabetic neuropathy contains an activator of Fas-regulated apoptosis that may contribute to the pathogenesis of diabetic neuropathy [17]. Pathogenetic mechanism of apoptosis and autoimmunity in both directions is also underscored by Mahoney and Rosen in their work [18].

Because of this markers of autoimmunity in diabetes require further testing and evaluation. No studies on the prevalence of autoantibodies ANA, AMA, ASMA, anti-LKM in people with diabetes have been carried out so far. It is not known whether the presence of these antibodies is related to the degree of metabolic balance of diabetes, the onset of complications and poor prognosis. We don't know if diabetic type 2 with ad on autoimmune phenomena takes different clinical course perhaps more aggressive one. In this context, the incidence of antibodies with autoimmune character should be assessed in patients with diabetes.

Objectives

The aim of study was to evaluate the incidence of immunological markers in subjects with type 2 diabetes: the anti-GAD, ANCA, ANA, AMA, ASMA, APCA, and LKM compared to healthy subjects. An additional objective of the study was to evaluate the correlation between the presence of selected markers of autoimmune diseases and the degree of metabolic control in subjects with type 2 diabetes.

Materials and Methods

The study included 50 subjects with previously diagnosed type 2 diabetes, who are under the care of the Department of Internal Diseases, Diabetology and Endocrinology, Warsaw Medical University. Inclusion criteria for the study included diabetes duration of at least 1 year, age range 40-75 years, BMI between 20-30 kg/m². The control group consisted of 21 healthy individuals without diabetes and prediabetes. All patients were subjected to the following procedures.

■ Medical history

A survey, with particular regard to data on family history of diabetes, duration of diabetes, recognized chronic microvascular complications: nephropathy, retinopathy, polyneuropathy, occurring autoimmune diseases and comorbidities, medication.

■ Physical examination

With particular emphasis on the following parameters: measurement of weight, height, waist circumference, hip circumference, calculation of BMI and WHR, blood pressure measurement, clinical examination directed to discover polyneuropathy -neurological examination of deep and superficial sensation (temperature, touch, pain), also vibration perception with tuning fork and monofilament test).

■ Laboratory tests of the following parameters

Glucose, glycated Hemoglobin (HbA1C), lipid profile, fasting C-peptide, creatinine, eGFR - calculated by the MDRD equation (Modification of Diet in Renal Disease), uric acid, aminotransferases (AST, ALT), morphology, TSH, micro albuminuria from 24h urine collection.

■ Immunological examination

Autoantibodies ANA, AMA, ASMA, APCA, LKM and anti-GAD titer in groups of people with diabetes.

■ The eye examination

Examination of the retina using indirect ophthalmoscopy.

Methods for Antibody Assays

■ The Presence of ANA, AMA, ASMA, LKM, APCA

Antibodies ANA, AMA, ASMA, APCA and LMC were analyzed by IIF according to the manufacturer's instructions - DakoCytomation, Glostrup, Denmark.

■ Confirmation of the Presence of ANA with Hep-2

The study was performed according to the manufacturer Euroimmun, Germany.

■ The presence of Anti-GAD

The study was performed according to the manufacturer - EUROIMMUN, Germany. The statistical analysis involved descriptive statistics, and a part was compared with the Chi-square test.

Results

The characteristics of the study groups are presented in **TABLE 1**. Numerical values are presented as mean ± Standard Deviation (SD). The prevalence of diabetes in first-degree relatives in the groups of patients is shown in **TABLE 2**.

Family history taking into account the prevalence of autoimmune diseases in the studied group of patients with diabetes type 2 is shown in **TABLE 3**. The incidences of co-occurrence

Table 1: Characteristics of groups of patients with type 2 diabetes	
Parameter	**Type 2 Diabetes Średnia (±Sd)**
Age (years)	62,08 (± 8,99)
Body Mass (kg)	81,74 (±12,80)
Growth (m)	1,69 (±0,10)
BMI (kg/m²)	28,31 (±2,13)
Waist Circumference (cm)	103,22 (±9,71)
Hip Circumference (cm)	107,05 (±6,253)
WHR	0,96 (±0,07)
Duration Of Diabetes (years)	11,33 (±9,77)

Table 2: Type of relationship in patients with a positive family history of diabetes.	
First-Degree Relative With Diabetes	**Percentage Of Group With Type 2 Diabetes**
Mother	24 %
Father	12 %
Siblings	4 %
Children	2 %

Table 3: Type of relationship in patients with a positive family history of the occurrence of other autoimmune disease	
First-Degree Relative Suffering From an Autoimmune Disease	**Percentage of Group with Type 2 Diabetes**
Mother	0 %
Father	0 %
Siblings	0 %
Children	8 %

of autoimmune diseases in people with type 2 diabetes are presented in **TABLE 4**. Prevalence of chronic diseases in patients with type 2 diabetes is shown in **TABLE 5**. Biochemical parameters are presented in **TABLE 6**. In the study group diabetes was chronically uncontrolled; HbA1c levels greater than 7% concerned 78% of respondents. The third stage of chronic kidney disease (with eGFR<60 ml/min/1.73 m²) was diagnosed in 20% of people with type 2 diabetes. The patients had unsatisfactory balance of lipid metabolism. Total cholesterol level higher than 175 mg/dl was observed in 52% of people with type 2 diabetes. Triglyceride level above 150 mg/dl was found respectively in 44% of subjects. The prevalence of diabetic retinopathy was found in 32% of subjects, nephropathy in 26% and neuropathy in 18%.

In the study group patients revealed antibodies of anti-GAD (16%), ANA (22%), ASMA and APCA, while no confirmed the presence of AMA antibodies and anti-LKM **(TABLE 7)**. Prevalence of autoantibodies in subgroups with particular micro vascular complications was not statistically increased and is presented in **TABLE 8**.

Discussion

In our study we marked selected autoantibodies in subjects with type 2 diabetes hospitalized in the Department of Internal Diseases, Diabetology and Endocrinology of Warsaw Medical University. Additionally the relation between the presence of antibodies and the degree of metabolic control and the presence of chronic complications in the form of retinopathy, nephropathy and peripheral neuropathy was searched. In the study group patients with type 2 diabetes the presence of antibodies anti-GAD, ANA, ASMA and APCA was revealed. In healthy individuals (16 women, 5 men, middle-aged, respectively 44 and 48 years) ANA, AMA, SMA, LKM, APCA antibodies were not found.

Anti-GAD are detected prior to clinical diagnosis and often persist for several years after diagnosis of type 1 diabetes [19], but can also disappear before the diagnosis [20]. Based on studies carried out in 2004 on a group of people of the population of North America and Europe, it is known that anti-GAD are present in 4.2% of people with newly diagnosed type 2 diabetes previously treated with oral antidiabetic agents. In this group of patients (newly diagnosed diabetes, showing the presence of anti-GAD), lower levels of fasting insulin, increased insulin sensitivity, lower HOMA are found. Lower concentrations of fasting insulin were accompanied by reduced early insulin response to oral glucose administration. In addition, patients with positive anti-GAD antibodies have higher HDL cholesterol levels and lower triglyceride levels. This group had a lower incidence of the metabolic syndrome compared to patients with type 2 diabetes not exhibiting the presence of anti-GAD (74.1% vs. 83.7%) [21].

The clinical trials conducted on a population of subjects with type 2 diabetes have associated anti-GAD with a lower BMI and smaller waist circumference. Patients with positive anti-GAD antibodies had higher fasting glucose, higher HbA1c and were often treated with insulin [22].

In the study group of people with type 2 diabetes, the presence of anti-GAD was associated with a lower HbA1c compared to those of type 2 diabetes without anti-GAD (7.5% vs. 8.74%) and lower average values of total cholesterol, LDL and triglycerides. The subjects with type 2 diabetes and positive anti-GAD antibodies had higher HDL cholesterol levels compared to individuals with type 2 diabetes not showing the presence of anti-GAD. Leslie et al., after noting

the presence of anti-GAD in subjects with type 2 diabetes changed the diagnosis for LADA [23].

Studied by us population with type 2 diabetes and positive anti-GAD was given another careful clinical evaluation. Most of these patients were treated with oral antidiabetic agents, only one person received insulin. Interview, anthropometric data and satisfactory glycemic control with the use of oral antidiabetic drugs confirm properly diagnosed type 2 diabetes in patients with autoantibodies (anti-GAD). Observations indicate that in some people with type 2 diabetes LADA should be diagnosed; there is also the possibility of the coexistence of two mechanisms of diabetogenic activity. The current classification of diabetes may, in the near future, require modification based on patient's detailed characteristics, phenotypic appearance, as well as the results of additional tests.

An interesting observation in our study includes the presence of additional antibodies of an autoimmune character in patients with type 2 diabetes, in the percentage of over 20%. Their role is not fully clear for the course to the disease, as well as their contribution in the pathogenesis of typical diabetes complications of both micro- and macroangiopathic character. The hint may come from examinations of subjects with systemic lupus erythematosus, wherein the presence of antinuclear antibodies is a marker of the disease, is involved in pathogenesis and is related to a defect in the cellular response to autoantigenes from, among others, apoptosis or necrosis. In people with type 2 diabetes, in the case of a worse metabolic control, we can expect increased apoptosis or necrosis dependent on microcirculation disorders and ischemic processes. Antinuclear antibodies may be the exponent of the above. Their presence can be exponent of immunological disorders or more likely because of their similar frequency in patients with type 2 diabetes, a marker of metabolic damage.

In previous scientific reports, the presence of ANA antibodies in healthy subjects was found at a frequency in the range of 4.2% - 22.6%, depending on the characteristics of the studied population [24-26].

Kaklikkaya et al. conducted a study to assess the prevalence of ANA in the Turkish population. Positive antinuclear antibodies were found in 16.11% of women and 13.68% men. The highest percentage of positive results was recorded in the age group 30-39 years (16.25%) and the lowest

Table 4: The incidence of co-occurrence of autoimmune diseases in people with type 2 diabetes.

Coexisting Autoimmune Disease	People With Type 2 Diabetes
Hashimoto's Thyroiditis	8 %
Graves' Disease	2 %
Psoriasis	2 %
Rheumatoid Arthritis	0 %

Table 5: The frequency of co-occurrence of chronic diseases in the study group patients

Coexisting Chronic Disease	People With Type 2 Diabetes
Hypertension	78 %
Ischemic Heart Disease	18 %
Myocardial Infarction In Past	8%
Chronic Kidney Disease	28 %
Hypercholesterolemia	76 %
Atherosclerosis	8%
Stroke In Past	8%

Table 6: Biochemical parameters in patients with type 2 diabetes

Parameter	Type 2 Diabetes Average (±SD)
Fasting Glucose (mg/dl)	118,76 (±26,03)
HbA1c (%)	8,74 (±2,177)
C-Peptide (ng/ml)	2,25 (±1,258)
Creatinine (mg/dl)	0,92 (±0,34)
eGFR (ml/min/1,73m^2)	82,539 (±25,56)
Uric Acid (mg/dl)	5,8 (±1,97)
Aspat (U/l)	29,86 (±24,40)
Alat (U/l)	32,2 (±25,0)
Total Cholesterol (mg/dl)	192,5 (±51,80)
Triglycerides (mg/dl)	174,64 (±102,17)
HDL (mg/dl)	46,72 (±10,02)
LDL (mg/dl)	109,0 (±102,17)
Microalbuminuria	28% (±51,70)

at the age of 70 years and above (12.72%). There was no statistically significant correlation between the presence of ANA and sex, age, place of residence, smoking and BMI [27].

In turn, the study of the Japanese population showed that among the 2,181 of studied people, 60 had a positive ANA - six of them were diagnosed with Sjögren's Syndrome (SS), in five Sjögren's Syndrome was suspected, and five had rheumatoid arthritis. In the group of patients with positive antibodies ANA, 50% had no symptoms of rheumatic disease [28].

The relation between ANA and diabetic neuropathy has been demonstrated in a study published in 2015. The authors investigated the presence of ANA antibodies in patients with diabetes and diabetic neuropathy, in patients with diabetes but with no neuropathy and in healthy

Table 7: The incidence of antibodies of selected autoantibodies in the study group patients.

Antibodies	Type 2 Diabetes (%)	Healthy People (%)
Anty- GAD	16	-
ANA	22	0 [1]
AMA	0	0
ASMA	4	0 [2]
APCA	10	0 [3]
Anty-LKM	0	0

[1]p = 0,019; [2]p = 0,35; [3]p = 0,13

Table 8: Microvascular complications in patients with different ANA profile

Type 2 Diabetes	ANA+	ANA-	p
Retinopathy	18,2%	35,9%	0,26
Nefropathy	36,3%	23,1%	0,37
Neuropathy	0%	23,1%	0,11

controls. The presence of ANA was 50-fold higher in patients with diabetes and neuropathy, compared with the control group [29]. It has also been shown that the presence of ANA antibodies in subjects with diabetes is associated not only with the development of autonomic neuropathy but cardiac complications as well [14].

In our study ANA were present in 11 of the studied subjects with type 2 diabetes. The presence of ANA was not associated with the occurrence of peripheral neuropathy, nor retinopathy and nephropathy.

Research performed on patient with autoimmune disorders points that systemic lupus erythematous and rheumatoid arthritis are connected with increased risk of cardiovascular diseases [30,31].

In the existing scientific reports there is no significant data on the incidence of APCA in people with type 2 diabetes. In our study, APCA occurred in 5 subjects. The presence of APCA was associated with the occurrence of peripheral neuropathy.

An important limitation of our study is its cross-cutting nature, which affects the assessment of mutual dependence. A major difficulty is the lack of standardization by age, although data obtained from the literature suggest that the presence of autoimmune antibodies shows no correlation with age. According to our results presence of autoimmune phenomena is quite common in diabetes type 2 and their role in whole pathogenesis is unclear. Group of patients with them should be observed and examined in prospective way.

Conclusion

The presence of anti-GAD in type 2 diabetic subjects may be a marker for LADA or specifically mark a group of patients with type 2 diabetes who are at risk of faster metabolic death of beta cells. The frequency of studied autoantibodies in patients with type 2 diabetes is high (at least 22%) but cannot be a marker used to isolate a group of patients at risk of developing diabetic micro vascular complications. AMA, ASMA, APCA and anti-LKM determination does not seem to be significant in the diagnosis of diabetes and its chronic complications.

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