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ORIGINAL ARTICLE

Autoimmunity markers in subjects with diabetes

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Abstract

Introduction. Diabetes is a growing social and epidemiological problem. Accordingly, the incidence of complications associated with diabetes can cause a persistent high percentage of diseases of the cardiovascular system, kidney and nervous systems, and impaired vision.

Objective. The aim of the study was to evaluate the incidence of immunological markers in patients with type 1 diabetes and those with type 2 diabetes: the anti-GAD, ANA, AMA, ASMA, APCA and LKM, compared to healthy people. Another objective of the study was to evaluate the correlation between their presence and the degree of metabolic control in both groups.

Materials and methods. The study comprised 100 subjects aged 25–75 years with a body mass index (BMI) between 20–30 kg / m2, hospitalized in the Department of Internal Diseases, Diabetology and Endocrinology, at the Medical University of Warsaw, with previously diagnosed diabetes who were assigned to one of 2 groups (50 subjects with type 1 diabetes and 50 subjects with type 2 diabetes). The control group consisted of 21 healthy individuals without the diagnosis of diabetes and 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 both groups of patients. Their presence was found in 30% of people with type 1 diabetes and in 16% of people with diabetes type 2. The presence of ANA antibodies was found in 24% of people with type 1 diabetes and 22% of people with type 2 diabetes. There was no correlation between the presence of ANA antibodies ANA and duration of diabetes. In the group of patients with type 1 diabetes, there was a correlation between the presence of ANA and the incidence of diabetic polyneuropathy. ASMA and APCA antibodies occurred with equal frequency in both studied groups (4% vs. 10%). There were no antibodies of AMA or anti-LKM in any of the patients.

Conclusions. Marking of ANA antibodies in patients with type 1 diabetes may be a marker used to isolate a group of patients at risk of developing diabetic neuropathy. The presence of anti-GAD in type 2 diabetes may be a LADA marker which specifically marks the group of patients with type 2 diabetes, in whom there is a faster metabolic death of beta cells. The current classification of diabetes is vague, and in the near future it should be modified based on specific patient characteristics, phenotypic appearance, as well as the results of additional tests. Determination of antibodies AMA, ASMA, APCA and anti-LKM does not seem to be significant in the diagnosis of diabetes and its chronic complications.

Key words

diabetes, anty-GAD,ANA, ASMA, APCA

Abbreviations

ICA – islet cell antibodies, IAA – insulin autoantibodies, 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, CAH – autoimmune chronic active hepatitis, VH – viral hepatitis, AH – autoimmune hepatitis, anti-IF – antibodies to Castle's intrinsic factor, AIG – autoimmune gastritis, BMI – Body Mass Index, WHR – waist-hip ratio, HbA1c – glycated haemoglobin, eGFR – estimated glomerular filtration rate, ss-DNA – single stranded DNA antibodies, HOMA – insulin resistance, SS – Sjögren's disease, anti-TPO antibodies – antibodies to the thyroid peroxidase, anti-Tg – antibodies to thyroglobulin, RA – rheumatoid arthritis

INTRODUCTION

The distinction between the two main conventional forms of diabetes in clinical practice is made mostly on the basis of clinical symptoms, prone to ketosis and the need for insulin. Sometimes, however, it is difficult to assign a clinical case clearly to one of the two basic forms of diabetes. There can be difficulties in making a correct diagnosis and, consequently, to select an optimal treatment. This is the result of objective epidemiological trends and the growing number of scientific reports that show how simplified was the previous understanding of etiopathogenesis of diabetes. In the last few years, it has been shown that autoimmune diabetes in adults occurs much more often than it previously thought [1].

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Chronic autoimmune process leading to the destruction of the B cells of pancreatic islets is a cellular response, and is preceded by several years of clinical manifestation of the disease [2, 3]. Antibodies associated with humoral response observed in type 1 diabetes is a marker of an 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 [4]. The prevalence of antibodies in type 1 diabetes compared to healthy volunteers [5, 6, 7] is shown in Table 1.

Table 1. Comparison of the prevalence of antibodies in the diabetic population

ANTIBODIES	TYPE 1 DIABETES	HEALTHY PEOPLE
ICA	60–80%	0.2–3%
IAA	>50%	1%
anty-GAD	80–90% in patients with newly diagnosed diabetes 70–80% during pre-clinical diabetes	2%
anty- IA-2	50–70%	1–2%

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, and the onset of complications and poor prognosis. In 2005, a study on evaluating the incidence of ANCA in patients with diabetes type 1 was published in which the authors also searched for a relationship between the presence of antibodies ANCA and the incidence of diabetic microangiopathy. No differences were observed in gender distribution, metabolic control parameters, the duration of diabetes, level of C-reactive protein, or retinopathy and nephropathy between the group with the presence of ANCA and a group of patients where these antibodies were not found. It should be pointed out that the study was limited to ANCA assessment [8].

Despite the diverse etiopathogenesis of diabetes and irrespective of the place in the classification of the disease, it always has the same micro- and macroangiopathic complications. Although conventional therapy lowers blood glucose levels and prevents acute metabolic complications in diabetic patients, it does not restore complete metabolic balance, which is related to a combination of the impact of metabolic, hormonal and hemodynamic disorders [3]. All these disorders are the result of exposure to hyperglycaemia. Risk factors for diabetic complications also include a genetic predisposition to their occurrence and the impact of the environment [2]. As already mentioned, the presence of various antibodies in a series of well-defined diseases is observed in other clinical situations involving the destruction of individual tissues. In the case of diabetes, which is a chronic disease, the presence of the aforementioned antibodies can be a marker of severe necrosis or apoptosis, which occurres due to damage in the course of metabolic disorders and microcirculation disorders. Activated immune process can also be an accelerating and independent in relation to the damage factor, factor in the pathogenesis of micro- and macroangiopathy. In this context, the incidence of antibodies with autoimmune character should be assessed in patients with diabetes.

OBJECTIVE

The objective of study was to evaluate the incidence of immunological markers in subjects with type 1 diabetes and those with type 2 diabetes: the anti-GAD, ANCA, ANA, AMA, ASMA, APCA, 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 1 diabetes and in subjects with type 2 diabetes.

MATERIALS AND METHODS

The study comprised 100 subjects with previously diagnosed diabetes, who were under the care of the Department of Internal Diseases, Diabetology and Endocrinology at the Medical University in Warsaw. Criteria for inclusion in the study were diabetes duration of at least one year, age range 25–75 years, BMI between 20–30 kg / m^2.

The study participants were divided into 2 groups depending on the type of diabetes:

Group 1: 50 subjects with type 1 diabetes;

Group 2: 50 subjects with type 2 diabetes.

The control group consisted of 21 healthy individuals without diabetes and prediabetes.

All patients were subjected to the following tests:

1) *physical examination* – a survey, with particular regard to data on family history of diabetes, duration of diabetes, occurring autoimmune diseases and comorbidities, medication;
2) *internal examination* – with particular emphasis on the following parameters: measurement of weight, height, waist circumference, hip circumference, calculation of BMI and WHR, blood pressure measurement
3) *laboratory tests of the following parameters* – glucose, glycated haemoglobin (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, microalbuminuria from 24h urine collection;
4) *immunological examination* – titer of autoantibodies ANA, AMA, ASMA, APCA, LKM and anti-GAD in groups of people with diabetes
5) eye examination – examination of the retina using indirect ophthalmoscopy.

Methods for antibody assays:

a) 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).
b) Confirmation of the presence of ANA with Hep-2.
c) Presence of anti-GAD.

The study was performed according to the manufacturer's instructions (Euroimmun, Germany).

Statistical analysis involved descriptive statistics, and a part was compared with the Chi-square test.

RESULTS

The group of subjects with type 1 diabetes and the group of subjects with type 2 diabetes did not differ significantly in terms of gender distribution. The characteristics of the study groups are presented in Table 2. Numerical values are presented as mean ± standard deviation (SD).

Table 2. Characteristics of groups of people with type 1 diabetes and type 2 diabetes

PARAMETER	TYPE 1 DIABETES średnia (±SD)	TYPE 2 DIABETES średnia (±SD)
AGE (years)	41.28 (± 13.37)	62.08 (± 8.99)
BODY MASS(kg)	70.22 (± 1.86)	81.74 (±12.80)
GROWTH (m)	1.72 (±0.09)	1.69 (±0.10)
BMI (kg/m^2)	23.64 (±3.01)	28.31 (±2.13)
WAIST CIRCUMFERENCE (cm)	84.27 (±10.95)	103.22 (±9.71)
HIP CIRCUMFERENCE (cm)	95.19 (±8.17)	107.05 (±6.253)
WHR	0.88 (±0.07)	0.96 (±0.07)
DURATION OF DIABETES (years)	13.48 (±12.96)	11.33 (±9.77)

Family history regarding the prevalence of diabetes in first-degree relatives in the groups of patients is shown in Table 3. Family history, taking into account the prevalence of autoimmune diseases in the studied group of patients with diabetes type 1 and type 2, is shown in Table 4. Autoimmune diseases are more common in first-degree relatives of subjects diagnosed with type 1 diabetes. The group of patients with type 1 diabetes revealed frequent co-occurrence of autoimmune diseases of the thyroid. Psoriasis appeared similarly in both groups. There was no incidence of RA in the studied group of patients with type 2 diabetes. Data are presented in Table 5. A higher incidence of chronic diseases was found in patients with type 2 diabetes (Tab. 6).

Table 3. Type of relationship in patients with a positive family history of diabetes

FIRST-DEGREE RELATIVE WITH DIABETES	PERCENTAGE OF GROUP WITH TYPE 1 DIABETES	PERCENTAGE OF GROUP WITH TYPE 2 DIABETES
MOTHER	14%	24%
FATHER	0%	12%
SIBLINGS	12%	4%
CHILDREN	0%	2%

Table 4. Type of relationship in patients with a positive family history of e occurrence of other autoimmune diseases

FIRST-DEGREE RELATIVE SUFFERING FROM AN AUTOIMMUNE DISEASE	PERCENTAGE OF GROUP WITH TYPE 1 DIABETES	PERCENTAGE OF GROUP WITH TYPE 2 DIABETES
MOTHER	14%	0%
FATHER	4%	0%
SIBLINGS	12%	0%
CHILDREN	2%	8%

Biochemical parameters in both groups are compared in Table 7. In both groups, diabetes was chronically uncontrolled, since up to 84% of patients with type 1 diabetes had Hb A1C levels above 7%. In the group of patients with type 2 diabetes, HbA1c levels greater than 7% concerned 78% of respondents.

Table 5. Incidence of co-occurrence of autoimmune diseases in people with type 1 diabetes and type 2 diabetes

CO-EXISTING AUTOIMMUNE DISEASE	PEOPLE WITH TYPE 1 DIABETES	PEOPLE WITH TYPE 2 DIABETES
HASHIMOTO'S THYROIDITIS	12%	8%
GRAVES' DISEASE	4%	2%
PSORIASIS	2%	2%
RHEUMATOID ARTHRITIS	2%	0%

Table 6. Frequency of co-occurrence of chronic diseases in people with type 1 diabetes and type 2 diabetes

CO-EXISTING CHRONIC DISEASE	PEOPLE WITH TYPE 1 DIABETES	PEOPLE WITH TYPE 2 DIABETES
HYPERTENSION	24%	78%
ISCHEMIC HEART DISEASE	6 %	18%
MYOCARDIAL INFARCTION IN PAST	2%	8%
CHRONIC KIDNEY DISEASE	14%	28%
HYPERCHOLESTEROLEMIA	42%	76%
ATHEROSCLEROSIS	4%	8%
STROKE IN PAST	0%	8%

Table 7. Comparison of biochemical parameters in patients with type 1 diabetes and in patients with type 2 diabetes

PARAMETER	TYPE 1 DIABETES average (±SD)	TYPE 2 DIABETES average (±SD)
FASTING GLUCOSE (mg/dl)	130.88 (±38.78)	118.76 (±26.03)
HbA1c (%)	9.75 (±2.644)	8.74 (±2.177)
C-PEPTIDE(ng/ml)	0.45 (±0.56)	2.25 (±1.258)
CREATININE (mg/dl)	0.88 (±0.65)	0.92 (±0.34)
eGFR (ml/min/1,73m^2)	106.42 (±33.05)	82.539 (±25.56)
URIC ACID (mg/dl)	3.62 (±1.5)	5.8 (±1.97)
ASPAT (U/l)	21.56 (±6.64)	29.86 (±24.40)
ALAT (U/l)	20.82 (±9.60)	32.2 (±25.0)
TOTAL CHOLESTEROL(mg/dl)	183 (±51.62)	192.5 (±51.80)
TRIGLYCERIDES (mg/dl)	111.24 (±55.28)	174.64 (±102.17)
HDL (mg/dl)	50.52 (±11.66)	46.72 (±10.02)
LDL (mg/dl)	110.26 (±43.31)	109.0 (±102.17)
MICROALBUMINURIA	14% (±62.35)	28% (±51.70)

Chronic kidney disease in the third period (with eGFR<60 ml / min /1.73 m^2) was diagnosed in 8% of people with type 1 diabetes and 20% of people with type 2 diabetes. Both groups of patients had an unsatisfactory balance of lipid metabolism. The level of total cholesterol above 175 mg/dl was observed in 50% of people with type 1 diabetes and 52% of people with type 2 diabetes. Triglyceride level above 150 mg/dl was found in 18% and 44% of subjects, respectively. Proteinuria was found in one person with type 1 diabetes with confirmed microalbuminuria, while among those with type 2 diabetes with confirmed microalbuminuria, 2 people had overt proteinuria. The prevalence of diabetic retinopathy was similar in both groups. It was found in 34% of subjects with type 1 diabetes and in 32% of people with type 2 diabetes.

Both groups revealed antibodies of anti-GAD, ANA, ASMA and APCA, while the presence of antibodies AMA and anti-LKM was not confirmed in any of the groups (Tab. 8).

Table 8. Incidence of antibodies of selected autoantibodies in patients with type 1 diabetes and in patients with type 2 diabetes

ANTIBODIES	TYPE 1 DIABETES (%)	TYPE 2 DIABETES (%)	HEALTHY PEOPLE (%)
Anty- GAD	30	16 [1]	-
ANA	24	22 [2]	0 [3,4]
AMA	0	0	0
ASMA	4	4	0 [5]
APCA	10	10	0 [6]
Anty-LKM	0	0	0

[1]p = 0.09; [2]p = 0.81; [3]p = 0.013; [4]p = 0.019; [5]p = 0.35; [6]p = 0.13

DISCUSSION

In this study, selected autoantibodies were observed in subjects hospitalized with type 1 diabetes and type 2 diabetes, and in healthy subjects. In addition, the relation between the presence of antibodies and the degree of metabolic control and the presence of chronic complications in the form of peripheral neuropathy was sought for. Both groups of subjects with diabetes type 1 and 2 revealed the presence of antibodies anti-GAD, ANA, ASMA and APCA. The presented study conducted in healthy individuals (16 women, 5 men, middle-aged, respectively 44 and 48 years) did not reveal ANA, AMA, SMA, LKM, APCA.

Anti-GAD are detected prior to clinical diagnosis and often persist for several years after diagnosis of type 1 diabetes [9], but can also disappear before the diagnosis [10]. Based on studies carried out in 2004 on a group of people in 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 was 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%) [11].

Different proposals were put forward in a paper published in 2015. The clinical trials conducted on a population of people 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 [12].

Scientific reports from 2000 found that patients with type 1 diabetes with a positive anti-GAD had poorer glycaemic control and a greater demand for insulin. In addition, patients with anti-GAD had slower conduction in median, ulnar and tibial motor nerves [13].

In the study group of subjects with type 1 diabetes, the presence of anti-GAD was associated with lower progression of diabetes, as evidenced by higher levels of C-peptide concentrations, lower fasting blood glucose, and lower HbA1c, compared to those with type 1 diabetes without the presence of anti-GAD (HbA1c 8.78% vs. 9.75%). No relation was found between the presence of anti-GAD and the balancing of lipid metabolism.

However, 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, have changed the diagnosis for LADA [14].

In the presented study, the population with type 2 diabetes and positive anti-GAD was given another careful clinical evaluation. Most of the patients were treated with oral antidiabetic agents, and only one person applied insulin. Interview, anthropometric data and satisfactory glycaemic 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 2 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 the current study includes the presence of additional antibodies of an autoimmune character, both in patients with diabetes type 1and type 2, in the percentage of over 20%. No statistically significant difference between these 2 groups was observed. Their role is not fully elucidated for the course to the disease, not their contribution in the pathogenesis of typical diabetes complications of both micro- and macroangiopathic character. A clue 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 related to a defect in the cellular response to autogenes from, among others, apoptosis or necrosis. In people with type 2 diabetes and type 1 diabetes, in the case of a worse metabolic control, increased apoptosis or necrosis dependent on microcirculation disorders and ischemic processes. Antinuclear antibodies may be the exponent of the above. Their presence can be the exponent of immunological disorders or, more likely, because of their similar frequency in patients with type 1 diabetes and type 2, 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 [15, 16, 17]. Kaklıkkaya et al. conducted a study to assess the prevalence of ANA in the Turkish population. Positive antinuclear antibodies were found in 16.11% of females and 13.68% of males. The highest percentage of positive results was recorded in the age group 30–39 years (16.25%) and the lowest 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 [18]. In turn, a study of the Japanese population showed that among the 2,181 studied

people, 60 had a positive ANA – 6 of them were diagnosed with Sjögren's syndrome (SS), in 5 Sjögren's syndrome was suspected, and 5 had rheumatoid arthritis. In the group of patients with positive antibodies ANA, 50% had no symptoms of rheumatic disease [19].

The relation between ANA and diabetic nephropathy has been demonstrated in a study published in 2015. This study investigated the presence of ANA antibodies in patients with diabetes, and diabetic neuropathy in patients with diabetes but with no neuropathy, and in healthy controls. The presence of ANA was 50 times higher in patients with diabetes and neuropathy, compared with the control group [20]. It has also been shown that the presence of ANA antibodies in people with diabetes is associated not only with the development of autonomic neuropathy, but also with cardiac complications [21]. ANA were present in 12 of the studied people with type 1 diabetes and 11 people with type 2 diabetes; but only in patients with type 1 diabetes the presence of ANA was associated with the occurrence of peripheral neuropathy. Such a relation has not been shown in patients with type 2 diabetes.

Earlier scientific publications already reported on the incidence of APCA with a frequency of 9% in people with type 1 diabetes diagnosed at the age of 30, while in healthy people aged 21–30, the incidence is estimated at 2.2% [22]. Karavanaki et al. showed that in a group of children with type 1 diabetes with the presence of anti-GAD with the duration of diabetes, there were more frequent anti-TPO and anti-Tg and APCA. It was found that anti-GAD can be used as a marker for the development of other autoimmune diseases in adolescents with type 1 diabetes [23].

Clinical studies published in 2011 evaluated the incidence of ANA, APCA, anti-TPO and IA-2 in patients with type 1 diabetes [24]. The average age of respondents was 13 years, average age at which diabetes was diagnosed was 8 years, and the average duration of diabetes – 5 years. Positive anti-GAD was found in 50% of the subjects and APCA in 10% of patients. Tested antibodies were more frequent in females. The time from the diagnosis was positively correlated with the presence of APCA, but demonstrated a negative correlation with the presence of anti-GAD. Therefore, patients diagnosed with type 1 diabetes at a younger age are less likely to have antibodies against GAD. The presence of anti-GAD is associated with older age at diagnosis of type 1 diabetes [25]. In the current study, APCA occurred with equal frequency in both groups of people with type 1 diabetes (5) and with type 2 diabetes (5). The presence of APCA was associated with the occurrence of peripheral neuropathy, both in subjects with type 1 diabetes and type 2 diabetes.

An important limitation of the presented 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. The importance of the presence of antibodies in the pathogenesis of diabetic complications requires further study and prospective evaluation of patient groups.

CONCLUSIONS

1. ANA antibodies determination in subjects with diabetes could be a marker used to isolate the group of patients at risk of developing diabetic complications, including diabetic neuropathy. Their specific meaning, however, requires a prospective assessment.
2. Determination of AMA, ASMA, APCA and anti-LKM does not seem to be significant in the diagnosis of diabetes and its chronic complications.
3. 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, in which there is a faster metabolic death of beta cells.
4. The current classification of diabetes is not precise and in the near future it should be modified based on specific patient characteristics, phenotypic appearance, as well as the results of additional tests.

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