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Relationship between the Level of Amylinemia and Albuminuria Categories in Patients with Latent Autoimmune Diabetes in Adults

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Abstract

BACKGROUND: β-cells of islets of Langerhans produce not only insulin but another hormone – amylin, whose role in the development and progression of chronic kidney disease (CKD) in patients with diabetes mellitus (DM) is not known for certain.

AIM: The aim of the study was to determine the relationship between amylinemia and albuminuria categories in patients with latent autoimmune diabetes in adults (LADA) and CKD.

METHODS: 89 patients with DM and CKD were examined, as well as 15 representatives of the control group. The patients were divided into three groups by the types of DM: 36 patients with LADA, 25 patients with classical type 1 diabetes mellitus (T1D), and 28 patients with type 2 diabetes (T2D). Serum amylin levels were measured using the enzyme-linked immunosorbent assay (ELISA) method.

RESULTS: In the group of patients with LADA, the amylin content was 9.0 times higher than in control ($p < 0.01$) and 6.8 times higher compared to classical T1D ($p < 0.01$); at the same time, it was 17.3% lower than in T2D group ($p < 0.05$). In patients with T1D, the level of amylinemia did not change, whereas in T2D group it was 10.8 times significantly higher compared to the control and 8.3 times higher than in the group of patients with classical T1D. The highest indicator was registered in patients with LADA2 phenotype. The level of amylin was increasing in proportion to the categories of albuminuria. Positive correlations were found between the content of amylin and insulin, C-peptide, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index and creatinine.

CONCLUSION: Serum amylin level significantly increases progrediently to the albuminuria categories in patients with LADA (especially in LADA2 phenotype) and T2D.

Introduction

The β-cell dysfunction in combination with insulin resistance is the key causes of the development of type 2 diabetes mellitus (T2D), while the processes of autoimmune aggression play a primary role in the manifestation of type 1 diabetes mellitus (T1D). Among the heterogeneous types of diabetes mellitus (DM), latent autoimmune diabetes in adults (LADA) is the most common and attracts the attention of scientists, the pathogenesis of which combines the main mechanisms of T1D and T2D. According to the updated classification of American Diabetes Association (ADA, 2021), LADA is classified as T1D, which develops in adulthood and has a slowly progressive course [1].

It is known that together with insulin, β-cells of the islets of Langerhans produce another hormone – amylin or islet amyloid polypeptide [2], which belongs to the procalcitonin family, normally penetrates the blood-brain barrier and, by binding to the neurons of the hunger center, is responsible for satiety [3].

Incontrovertible evidence from numerous studies indicates a clear link between pancreatic amyloid and the development of classical types of DM [4], [5], [6].

At the same time, the role of hyperammonemia in the development and progression of LADA, which is characterized by signs of both types of diabetes, remains unnoticed by researchers.

Gong *et al*. found that in patients with histologically confirmed diabetic kidney disease (DKD), amylin was mainly distributed in the expanded mesangial zone, Kimmelstil-Wilson nodes, Bowman capsule and in blood vessels, and the degree of deposits was directly proportional to the severity of the underlying disease. The frequency of mesangial proliferation, lesions, and glomerular sclerosis was higher in patients with DKD and amylin deposition [7].

Despite all of the above, the effect of amylin levels on the course of diabetes is still controversial and ambiguous, and the role of hyperammonemia in the development and progression of micro- and macrovascular complications, in particular DKD, remains unnoticed by researchers.

The objective of the study was to clarify the relationship between the level of amylinemia and albuminuria categories in patients with latent

autoimmune diabetes in adults compared to classical types of diabetes.

Methods

Participants

Before the start of the study, the approval of the Commission on Biomedical Ethics was obtained regarding compliance with the moral and legal rules for conducting medical scientific research (clinical) of Bukovinian State Medical University (Minute No. 2 of October 17, 2019). To be included in the study, all subjects signed informed consent forms.

89 patients with DM who were treated at the Chernivtsi Regional Endocrinology Center were examined (the average age of patients was 44.4 ± 1.36 years; men – 43, women – 46), as well as 15 representatives of the control group. The patients were divided into three groups, two of which included 110 patients with T1D – 25 patients with classical T1D and 36 people with LADA. The third group consisted of 28 patients with T2D.

The age of manifestation of LADA was 36.6 ± 1.59 years, while the classical T1D was 16.3 ± 1.25 years and T2D was 44.4 ± 1.11 years. At the time of the study, the duration of the disease in LADA patients was 5.5 ± 0.89 years, in patients with T1D – 19.0 ± 1.86 years, in patients with T2D – 11.0 ± 1.11 years. The diagnosis of CKD in most patients with LADA was established less than 3 years after the onset of the disease, which are radically different from the results obtained with T1D (7.6 years) and slightly different from T2D (1.8 years, respectively).

Diagnosis

The diagnosis of DM was established according to the recommendations of ADA (2021) [1], the diagnosis of LADA – according to the recommendations of the Immunology of Diabetes Society (IDS, 2005) [8] and the consensus proposed by Buzzetti *et al*. (2020) with the expansion of the characteristics of LADA, which included the age of manifestation – more than 30 years; family/personal history of autoimmunity; reduced frequency of metabolic syndrome compared with T2D – low rates of insulin resistance, body weight, blood pressure, and dyslipidemia compared with T2D; no difference in cardiovascular consequences for patients with LADA and patients with T2D; slower decrease in C-peptide levels than with T1D; positivity for antibodies to glutamic acid decarboxylase (antiGAD) as the most sensitive marker (less often than other antibodies – ICA, IA-2A ab, ZnT8A and tetraspanin-7); and no need for insulin therapy at the onset of diabetes [9].

According to the main phenotypes, LADA patients were divided into two groups: LADA1 included 19 individuals with high titers of antiGAD ≥180 U/ml and LADA2, whose number was 17 individuals with low antiGAD titers – from 18 to 180 U/ml [10].

The CKD diagnosis was established according to the recommendations of KDIGO (2012) [11]. GFR was determined by the CKD-EPI formula according to the recommendations of KDIGO (2012) [11]. Studies of antiGAD and IA-2a ab were carried out by the enzyme immunoassay using sets of "Diametra S. L. R." (Italy). The amylin content was determined by enzyme immunoassay using Elabscience kits (USA) (normal values 4.0–25.0 pmol/l). The category of albuminuria was determined by the indicators of microalbuminuria (MAU) and the ratio of albumin to creatinine (RAC) in urine using the kits of the NGO NPL "Granum" (Ukraine).

Statistical analysis

Statistical processing of the obtained data was carried out in Statistica version 13.3 and Microsoft Excel 2016 using the Wilcoxon-Mann–Whitney U-test. The results were considered statistically significant at $p < 0.05$.

Before testing the statistical hypotheses, the asymmetry and excess coefficients were determined using the Khan-Shapiro–Wilk test to analyze the normality of the distribution of values in randomized samples. Student's *t*-test was used only in the case of a normal distribution of the equality of the general variances of the compared samples, which was checked using Fisher's F-test. In other cases, the non-parametric Mann–Whitney rank test was used to compare the results. Analysis of variance was used to compare several groups.

Results

After determining the serum level of amylin depending on the type of DM, it was found (Table 1) that in the LADA group, the amylin content in the blood serum was 9.0 times higher ($p < 0.01$) relative to the control group and 6.8 times higher than in the classical

Table 1: The level of amylin in the blood serum of patients with diabetes mellitus and chronic kidney disease, depending on the type of underlying disease

Groups	Amylin, pmol/l	Probability, p
Control group, n = 15	12.71 ± 1.773	
T1D, n = 25	18.33 ± 2.631	<0.05
T2D, n = 28	145.85 ± 29.137	<0.01
LADA, n = 36	120.55 ± 27.015	<0.01
LADA1, n = 19	75.20 ± 30.410	<0.01
LADA2, n = 17	171.20 ± 43.750	<0.01

p – reliability of changes relative to control; T1D – type 1 diabetes mellitus; T2D – type 2 diabetes mellitus; LADA – latent autoimmune diabetes in adults; LADA1 – phenotype 1 of latent autoimmune diabetes in adults; LADA2 – phenotype 2 of latent autoimmune diabetes in adults.

T1D, respectively (p < 0.01), at the same time it was 17.3% lower than that in the T2D group (p < 0.05). In classical T1D it was higher than in control by 44.2% (p < 0.05), while in patients with T2D – 10.8 times higher than in control group (p < 0.01) and 8.3 times higher than in the group of patients with classical T1D, respectively (p < 0.01).

In the distribution of patients with LADA into phenotypes, in patients with LADA1, the serum level of amylin was 5.9 times higher relative to the control (p < 0.01), 4.1 times higher relative to classical T1D (p < 0.01), but almost half as low as in T2D (p < 0.01). In LADA2 group, the above indicator exceeded that in the control group by 13.5 times (p < 0.01), in the group of classical T1D – by 9.3 times (p < 0.01), and in patients with LADA1 – by 2.3 times (p <0.05).

Due to the cosecretion of insulin and amylin, it is obvious that the content of the latter is significantly higher in T2D and LADA (especially LADA2), compared with classical T1D.

In patients with LADA, an interdependence was found between the content of amylin and insulin (r = 0.64; p = 0.000), C-peptide (r = 0.74; p = 0.000), and the HOMA-IR index (r = 0.54; p = 0.001), which indicates the role of insulin resistance, as well as hyperamylinemia caused by it, in the development and progression of this subtype of diabetes. In case of classical T1D, there was no probable relationship between the indicators. In T2D, probable dependence was recorded between the indicators of amylin and C-peptide (r = 0.83; p = 0.000) and the HOMA-IR index (r = 0.42; p = 0.027), which confirms the relationship between increased synthesis of amylin and insulin in this category of patients.

Regarding the dependence of the level of amylin on the degree of albuminuria (Table 2), we found the following trends: The lowest indicator was recorded in patients with category of albuminuria A1 – it was 6.5 times higher than in the control group (p < 0.05) and 2.9 times higher than in A3 (p < 0.05); in patients with category A2, the level of amylin increased 9.0 times compared to the control group (p < 0.05) and did not change compared to the indicator in the group of persons with category A1; in patients with category A3, the level of the studied indicator was 18.9 times higher relative to control (p < 0.01). In other cases, no significant difference between the indicators was found.

Table 2: Indicators of amylinemia in patients with diabetes mellitus, depending on the category of albuminuria

Indicator	Amylin, pmol/l	Probability, p
Control group, n = 15	12.71 ± 1.773	
Albuminuria categories		
A1, n = 47	83.20 ± 17.918	<0.05
A2, n = 35	114.30 ± 27.150	<0.05
A3, n = 7	240.63 ± 77.657	<0.01

p – Probability of changes in control.

Taking into account the results obtained, it can be concluded that the level of amylin increased in proportion to the albuminuria categories.

In patients with CKD and DM, significant positive moderate correlations between the content of amylin and insulin (r = 0.64; p = 0.000), C-peptide (r = 0.68; p = 0.000), HOMA rate (r = 0.51; p = 0.001), and low – between the level of amylin and serum creatinine (r = 0.29; p = 0.005) were revealed.

At the same time, in the group of patients with T2D, statistically significant direct correlations were established between serum amylin and serum creatinine (r = 0.73; p = 0.000) and GFR (r = 0.57; p = 0.001), which indicates the role of hyperammonemia in the development of CKD in this category of patients.

Discussion

It is known that insulin resistance provokes not only an increase in the production of insulin by β-cells of the pancreas, but also amylin, which, under conditions of hypersecretion, is capable of aggregation and deposition in the form of amyloid protein, in particular in pancreatic tissues and microvascular bed of various organs and systems. This is the very mechanism that suggests the role of hyperammonemia in the development of DKD [12]. Nevertheless, it is important that amylin oligomers have a greater cytotoxic effect, in contrast to the formed deposits [13].

Numerous clinical and experimental studies have proved that in excess, amylin causes apoptosis of mesangial cells and increases the permeability of endothelial cells of the nephron. Amylin deposits were found in the kidneys and heart of patients with T2D, as well as in brain tissues during the development of dementia disorders [14], [15], [16], [17], [18], [19], [20], [21], [22], [23].

The results of our study indicate the probable role of hyperammonemia in the development of CKD at DM in patients. Kidney damage in this case can occur by binding amylin to procalcitonin and AGE22 (receptor for advance glycosylation endproducts) receptors on the podocyte membrane. RAGE22, in turn, activates p21ras, mitogen-activated protein kinase, nuclear kB-factor, and CDC42/Ras pathways. Stimulation of RAGE22 induces the expression of endothelial vascular growth factor, which causes endothelial cell hyperpermeability, disrupts the expression of intracellular adhesion molecule-1, promotes adhesion and inflammation of macrophages, activates nuclear kB-factor and increases the expression of pro-inflammatory cytokines – TNF-α, IL-1β, and IL-6, which leads to podocyte damage and the occurrence of proteinuria [7].

In turn, in DKD, redox stress caused by hyperglycemia, lipotoxicity and glycotoxicity contributes to the accelerated formation of amylin deposits by

cross-linking oligomers with each other by the end products of glycosylation. Thus, strict metabolic control and reduction of insulin resistance significantly inhibit the formation and accumulation of amylin deposits, which is very important for the prevention of CKD development and progression in DM.

Conclusion

In patients with diabetes mellitus, serum amylin levels significantly increase progrediently to the albuminuria categories. Serum amylin levels were significantly higher in patients with chronic kidney disease and latent autoimmune diabetes in adults (especially in the LADA2 phenotype), as well as type 2 diabetes, compared with classical type 1 diabetes.

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