CLINICAL AND PATHOGENETIC ROLE OF HEPATITIS C VIRUS IN THE DEVELOPMENT OF AUTOIMMUNE PROCESSES OF THE PANCREAS

M. A. Derbak

Uzhgorod National University, Ukraine

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Today more attention has been paid to the study of the immune system not only in patients with type 1 diabetes, but also with type 2 diabetes. In many cases, determination of the diabetes type does not cause any diagnostic doubts. It is believed that type 1 diabetes occurs in young people and is characterized by acute onset with a development of ketoacidosis and the rapid development of clinical manifestations. Type 2 diabetes is more typical for most of the patients with obesity, often elder, with compensation of carbohydrate metabolism achieved by the diet and prescription of hypoglycohaemic drugs per os [1].

Research groups from around the world have received the data on the existence of the specific diabetes type in the adult population in the past few years, particularly Latent Autoimmune Diabetes in Adults (LADA) [10, 12].

Autoimmune process in LADA has a number of features. The main types of autoantibodies in patients with LADA — glutamate decarboxylase antibodies (GADA) and islet cells antibodies (cytoplasmic components of β-cells) (ISA), GADA being detected more often than ISA [11]. The rate of decrease in the secretory function of β-cells in LADA is slower than in type 1 diabetes, residual secretion is preserved for many years [2]. Insulin requirement is developed in a few years in more than 80% of patients with LADA, and there is a risk of other organ-specific autoimmune diseases [7].

The study of hepatitis C (HCV) is still being not less important today. This is due to its wide spread and the global trend to the increasing infection of population. According to WHO, there are about 170 million HCV-infected in the world. Progression of chronic hepatitis may occur in more than 60% of patients with acute hepati

The finding of HCV replication outside the liver has been one of the most important recent discoveries. It is possible to consider HCV-infection as a systemic (generalized) infectious disease [5, 14]. HCV persistence often leads to abnormalities in the immune system. The main manifestations of these violations are in the appearance of autoantibodies in blood serum, in many cases accompanied with autoimmune lesions of the relevant organs. Autoantibodies are found in 18-91% of patients with chronic hepatitis C (CHC). In general, upon CHC different antibodies can be detected, namely antinuclear (ANA in 8-63% of patients), smooth muscle (SMA in 5-65%), antimitochondrial (AMA in 4-8%), cardiolipin (22-34%), antithyroid (10-20%), antiplatelet, antibodies to DNA and nucleoproteins, to liver and kidneys microsomes (LKM-1 in the 0-20%), epithelial antigens, gastric parietal cells (GPC in 32%) and cytoplasmic antigens of neutrophils. Such a wide spectrum of autoantibodies leads to a higher rate of autoimmune diseases in patients with CHC [3, 4, 6]. Some researchers believe that it is necessary to test patients with CHC for the presence of autoimmune processes due to the need for a special approach to their treatment [9]. Conversely, patients with certain autoimmune diseases should be screened for infection with HCV, since systemic effect of the virus, which is now more than obvious, causes autoimmune manifestations [15].

Therefore, a number of studies on the role of HCV in the development of various autoimmune reactions have been conducted. However, the question remains whether the HCV-infection affects the course of autoimmune response directed to the β-cells of the pancreas, the secretion of insulin, which has determined our interest in this problem.

Aim of research is to examine the value of HCV in the initiation of autoimmune processes directed to β-cells of the pancreas, accompanied with a decrease in insulin secretion in patients with type 2 diabetes.

Materials and methods. We examined 400 patients aged from 31 to 70 (mean — 54.1±1.1): 247 (61.7%) men and 153 (38.3%) women were treated in the Departments of Endocrinology and Gastroenterology of Transcarpathian Regional Clinical Hospital n. a. A. Novak and Department of Therapy of Ministry of Home

Affairs Hospital in the Transcarpathian region during 2011-2013. Besides standard examination, in accordance with the protocols of medical care for the patients of endocrinology and gastroenterological profile, the study included: determination of C-peptide, GADA and ICA in serum by enzyme immunoassay, determination of viral load by PCR, analysis of data from medical history and clinical course of type 2 diabetes. The degree of diabetes compensation was set by the level of glycosylated hemoglobin (HbA1c).

We formed 2 groups of patients: the first studied group consisted of 125 HCV-infected patients with type 2 diabetes, the second comparison group consisted of 275 patients with type 2 diabetes without HCV. The first group was divided into: 1a subgroup (n=49) — patients with high viral load RNA HCV⩽4x105 IU/ml and 1b subgroup (n=76) — patients with low viral load RNA HCV<4h105 IU/ml. The groups were representative by gender, age and duration of diabetes.

Assessing the significance of differences in relative values in independent samples was carried out by the test of the null statistical hypothesis of equality of relative frequencies in two samples and in bilateral exact Fisher criteria with the use of program Statistica 8.0 for Windows. The difference was considered valid at $p<0.05$.

Results and discussion. Secretion of C-peptide in diabetic patients depends on several factors, including compensation of diabetes and glucose toxicity, state of insulin resistance in the organism, disease duration, age of the patient, the therapeutic approach, some aggressive environmental factors, concomitant diseases, etc. Therefore, we hypothesized that the basic laws of the functional state of β-cells could be traced by analyzing a group united by one main factor influencing the target cell, in this case — HCV.

By studying the levels of C-peptide secretion, we found that its reduced secretion significantly more frequently was detected in patients with type 2 diabetes with HCV, than in uninfected, namely in 70.4% of patients versus 6.2% ($p<0.001$). In the subgroup of patients with a high viral load of HCV C-peptide was below norm in 83.6% of cases, while in the subgroup with low viral load — only in 61.8% of cases.

Considering the ability of HCV to initiate autoimmune processes in the organism, this study assessed the frequency of detection of autoimmune antibodies in these groups of patients. Highly specific markers of autoimmune hepatitis/crossed syndrome (anti-LKM-l, anti-SLA and anti-LC-l) were not detected in any patient. According to the current data, GADA and ICA are highly specific markers of autoimmune processes in β-cells in immunological studies. In the available literature we failed to find data on the effect of HCV load level on the incidence of GADA and ICA. However, in the works by F. Cassani et al., B. D. Clifford et al. there are indications of the detection of autoantibodies to liver cells only on the background of viral replication [8, 13]. So, it was important for us to clarify the correlation of markers of autoimmune aggression, the main links of homeostasis of carbohydrate metabolism and HCV viral load.

Analysis of clinical material showed statistically significantly higher incidence of GADA (41.6%) in patients with type 2 diabetes with CHC as compared to the patients without CHC (5.5%) ($p<0.001$). The frequency of GADA depended on the degree of viral load. Thus, in patients with high viral load, it amounted to 65.3%, and in the low — 26.3%. According to the above-mentioned division, the incidence of ISA in serum was studied in the same groups. The conducted analysis showed a higher incidence of ICA in patients of group 1 (16.8%) as compared to the patients of group 2 (1.8%) ($p<0.001$). The number of ICA-positive patients was also significantly greater in the group with high viral load as compared to the patients with low viral load (22.4% and 13.1%, respectively).

In 17 patients of group 1 in serum both types of antibodies were detected, which comprised 13.6% of all the HCV-infected. Simultaneous determination of GADA and ICA in patients with type 2 diabetes more fully characterized autoimmune processes in target cell and significantly increased the probability of prognosis of its destruction and, consequently, insulin deficiency, as evidenced by the great number of studies. Therefore, the frequency of the simultaneous presence of two types of autoantibodies was also analyzed according to the viral load.

The rate of the two types of antibodies was higher in patients with high viral load (22.4%) than low one (7.9%) ($p<0.05$). None of the patients with CHC had ICA and GADA at the same time. This fully corresponded to our earlier analysis, which showed a higher incidence of ICA and GADA separately in the serum of diabetic patients infected with HCV (Table 1).

Table 1

Correlation between viral load, rate of GADA and ICA detection and C-peptide in patients with type 2 diabetes

Groups of patients	Presence of autoimmune antibodies			C-peptide	
	GADA	ICA	GADA+ICA	below norm	norm
1 group (n=125) type 2 diabetes + CHC	52 (41.6%)	21 (16.8%)	17 (13.6%)	88 (70.4%)	37 (29.6%)
1a subgroup (n=49) type 2 diabetes + HCV high viral load	32 (65.3%)	11 (22.4%)	11 (22.4%)	41 (83.6%)	8 (16.3%)
1b subgroup (n=76) type 2 diabetes + HCV high low viral load	20 (26.3%)	10 (13.1%)	6 (7.9%)	47 (61.8%)	29 (38.1%)
2 group (n=275) type 2 diabetes without CHC	15 (5.5%)	5 (1.8%)	0	17 (6.2%)	258 (93.8%)

Important data were obtained by analyzing the frequency of detection of GADA and ICA together. GADA were determined more commonly in all the groups. This is consistent with the literature data that GADA with high specificity and information content are of the most predictive value for the development of secretory β-cell insufficiency.

Study of the prognostic significance of immunological parameters revealed a high degree of correlation between the presence of GADA and ICA levels in blood serum and the subsequent development of insulin dependence in HCV-infected patients, as evidenced by a decrease of the C-peptide level in these patients.

У процесі дослідження в цих же групах хворих, був проведений комплексний аналіз результатів визначення HbA1c, що специфічно характеризує компенсацію та ступінь важкості ЦД. Аналіз показав, що, в цілому, хворі 1 і 2 груп мали приблизно однакові рівні компенсації вуглеводного обміну, але трохи відрізнялися за ступенем важкості ЦД 2 типу. Так, серед хворих із ХГС важку ступінь ЦД мали 49,6% пацієнтів, а без вірусу

— 26,5% (табл. 2). Рівень реплікативної активності HCV на ступінь важкості діабету не впливав.

In studying the same groups of patients, a comprehensive analysis of the determination of HbA1c was carried out, which specifically described the compensation and the severity of diabetes. The analysis showed that, overall, patients of the 1 and 2 groups had almost the same level of compensation of carbohydrate metabolism, but slightly differed in the severity of type 2 diabetes. For example, among patients with CHC, severe diabetes was in 49.6% of patients, and without virus — in 26.5% (Table 2). The level of HCV replicative activity had no effect on the severity of diabetes.

Table 2

Correlation between viral load, severity of diabetes and the level of diabetes compensation

Groups of patients	**Degree of severity of type 2 diabetes**			**Level of compensation of carbohydrate metabolism**		
	Slight	Moderate	Severe	Compensated HbA_{1c} 6,0–6,5%	Subcompensated HbA_{1c} 6,6–7,0%	Decompensated HbA_{1c} >7,0%
1 group (n=125) type 2 diabetes + CHC	14 (11.2%)	49 (39.2%)	62 (49.6%)	7 (5.6%)	25 (20%)	93 (74.4%)
1a subgroup (n=49) type 2 diabetes + HCV high viral load	3 (6.12%)	19 (38.8%)	27 (55.1%)	0	5 (10.2%)	44 (89.8%)
1b subgroup (n=76) type 2 diabetes + HCV high low viral load	11 (14.5%)	27 (35.5%)	38 (50%)	7 (9.2%)	20 (26.3%)	49 (64.5%)
2 group (n=275) type 2 diabetes without CHC	95 (34.6%)	107 (38.9%)	73 (26.5%)	23 (8.4%)	60 (21.8%)	192 (69.8%)

Upon the evaluation of compensation between groups of patients with high and low HCV viral load, to the HbA1c level pointed out the decompensation of carbohydrate metabolism in the majority of patients with a high load, namely in 89.8% (44 of 49), and no patient in this group of diabetes had compensated diabetes. In patients with low HCV viral load, decompensated carbohydrate metabolism was in 64.5% (49 of 76) of patients and compensated — in 9.2% (7 of 76), respectively.

Thus, it is evident that replicating HCV activity in patients with type 2 diabetes in combination with CHC affects the compensation of carbohydrate metabolism, decreasing its (Table 2).

By carrying out a comprehensive analysis, we can conclude that HCV-infection caused autoimmune response to β-cells of the pancreas in 41.6% (52 of 125) of patients with type 2 diabetes, with a large number of patients in the group with high replicative activity. There was also a significant effect of high viral load on the degree of compensation of diabetes, as there was no patient with compensated diabetes among the patients with high HCV load.

Conclusions:

1. High replicating HCV activity is accompanied with the decreasing compensation of carbohydrate metabolism in patients with type 2 diabetes.
2. GADA and ICA are significantly more often registered in patients with type 2 diabetes combined with CHC than in patients without CHC (41.6% and 5.5% compared to 16.8 and 1.8%).
3. HCV may be the initiating factor in the development of autoimmune reactions directed to the β-cells of the pancreas in patients with type 2 diabetes, with following insulin dependence.

Upon high viral load and poor compensation of diabetes on the background of traditional hypoglycemic therapy, determination of GADA and ICA should be included to the comprehensive examination of patients.

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Clinical and pathogenetic role of hepatitis C virus in the development of autoimmune processes of the pancreas

M. A. Derbak

Uzhgorod National University, Ukraine

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Results of the examination of 400 patients with type 2 diabetes are presented in the paper. We formed 2 groups of patients, the first study group consisted of 125 patients with type 2 diabetes infected with hepatitis C virus (HCV), and the second comparison group consisted of 275 patients with type 2 diabetes without HCV. It was revealed that in patients with type 2 diabetes with HVC, GADA and ICA were detected significantly more often (41.6% and 5.5%) than in patients without HCV (16.8% and 1 8%). It was also shown that the HCV high replicating activity was accompanied by deterioration of the compensation type 2 diabetes and decreased insulin secretion. Thus, it was proved that HCV might be a factor in initiating the development of autoimmune reactions directed to the pancreatic β-cells in patients with type 2 diabetes.