SHORT COMMUNICATIONS

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THE IMPACT OF GENETIC FACTORS ON THYROID HORMONES METABOLISM IN PATIENTS WITH DIABETIC KIDNEY DISEASE

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One out of eleven adults in the world has diabetes mellitus. 25% of them develop diabetic kidney disease. Thyroid hormones are involved in the regulation of almost all physiological processes in the body, including renal function. The aim of the research was to study the dependance of biochemical markers of renal function in patients with diabetic kidney disease on C/T polymorphism in the DIO1 gene. To assess the dependence of biochemical markers of renal function on the C/T polymorphism in the DIO1 gene, the following groups has been formed: 19 patients with CC genotype, 69 individuals - with CT and 14 ones- with TT genotypes. Content of urea and cleatinine in plasma, eGFR, as well as microalbumine and creatinine content in urine were sagnificantly higher in patients with TT genotype than in group of patients with CC genotype and control group ($P < 0.05$). Presence of the T allele in genotype is associated with violation of thyroid hormones metabolism with the development of nonthyroidal illness syndrome. Carriers of T allele with diabetic kidney disease had significantly worse biochemical indices of renal function, that indicates the dependence of these markers on DIO1 polymorphism.

K e y w o r d s: C/T polymorphism in the DIO 1 gene, nonthyriodal illness syndrome, thyroid status assessment, biochemical indices of renal function, diabetic kidney disease.

The prevalence of diabetes mellitus (DM) is 9.3% worldwide (1 out of 11 adults), 90% of them have type 2 DM [1]. Approximately 25% of individuals with DM develop diabetic kidney disease (DKD), which refers to chronic kidney disease (CKD) supposed to be caused by diabetes [2]. Thyroid hormones are involved in the regulation of almost all physiological processes in thre body, including renal function. Decrease in thyroid hormones levels is associated with reduced blood flow to kidneys and decreased glomerular filtration rate along with alteration of tubular reabsorption [3].

70% of hospitalized patients develop nonthyriodal illness syndrome (NTIS) or "low T_3 syndrome" caused by decreased metabolism of thyroid hormones in peripheral organs and tissues that normally is provided by special enzymes, called deiodinases. NTIS is characterized by low normal level of triiodthyrinine (T_3), high normal level of thyroxine (T_4) and inappropriately normal thyroid stimulating hormone (TSH) level in blood serum, but severe tissue hypothyroidism [4]. Deiodianse type 1 (D1) is an enzyme that is active in liver and kidneys and plays an important role in activation of prohormone T_4 to 5 times more active T_3 [5, 6]. Mechanism of the reduction of this enzyme activity in patients with diabetes mellitus is multifactorial but can be aggravated by genetical factors as well [7].

Expression of D1 is regulated by *DIO1* gene. We chose C/T polymorphism at position 785 in the *DIO1* gene of complementary DNA for analyzing the influence of genetic factors on thyroid hormones metabolism in patients with DKD [8].

Previous studies reported that C/T polymorphism in the *DIO1* gene is related to the impairment of thyroid hormones metabolism: presence of minor T-allele in the genotype is associated with an increase in the reverse T_3 (rT_3) in plasma, an increase in rT_3/T_4 ratio and a decrease in the T_3/rT_3 ratio [4, 9].

The aim of the research was to study the dependence of biochemical markers of renal function on C/T polymorphism in the *DIO1* gene in patients with diabetic kidney disease.

Material and Methods

The research was conducted on the basis of clinical and diagnostic laboratories of the Department of Internal Medicine, the Department of Medical Biology, Genetics and Pharmaceutical Botany of the Bukovinian State Medical University, the Regional Municipal Institution "Regional endocrinological clinic", the Chernivtsi Regional Hospital of Veterans of War.

The C/T polymorphism in the DIO1 gene was studied in 102 patients with DM type 2 complicated by DKD in stage of microalbuminuria and 97 healthy subjects formed the control group.

The average age of patients was 52.5 ± 8.8 years: 35 patients (34.3%) were women, 67 (65.7%) patients – men. The control group included 97 practically healthy persons at the age of 48.90 ± 7.96 years: 58 persons (59.8%) were men and 39 – women (40.2%).

In the following study the principles of bioethics were respected: the main provisions of the European Convention on Human Rights and Biomedicine (04.04.1997), GCP (1996), Helsinki Declaration of the World Medical Association on the Ethical Principles of Human Medical Scientific Research (1964–2000) and the Ministry of Health of Ukraine Order No 281 dated back to 01.11.2000. The study protocol and Informed Consent form for patient was approved by the Ethics Committee of the Bukovinian State Medical University, Ukraine (protocol No 9, February 15, 2011).

Inclusion criteria: informed consent of the patient to participate in the study, diagnosed arterial hypertension, combined with abdominal obesity, a violation of carbohydrate metabolism in the form of type 2 diabetes mellitus, dyslipidemia, CKD that was diagnosed as renal dysfunction characterized by estimated glomerular filtration rate (eGFR) of ≤90 ml/min that persisted for more than three months with proteinuria.

Exclusion criteria: secondary arterial hypertension, hypothyroidism, thyrotoxicosis, decompensated kidney and liver damage, chronic heart failure above FC III, left ventricular ejection fraction up to 45%, acute cerebrovascular accident and acute coronary syndrome less than 3 months before the study, mental disorders, pregnant women, lactating, any chronic diseases in the acute stage and acute inflammatory processes, other comorbid diseases in the stage of decompensation or acute conditions capable to influence research results.

Metabolic syndrome was determined according to the recommendations of the International Diabetic Federation (IDF), 2005.

To assess the dependence of biochemical markers of renal function on the C/T polymorphism in the DIO 1 gene, the following groups has been formed: 19 patients with CC genotype, 69 individuals – with CT and 14 ones – with TT genotypes.

C/T polymorphism in the DIO1 gene was studied by isolation of genomic DNA from peripheral blood leukocytes, after that amplification of the polymorphic area in the state of polymerase chain reaction (PCR) was performed on the programmed PCR thermal cyclers Amply-4L (Biocom, RF) at individual temperature response. Reagents "DNA sorb-V" option 100 were used for DNA isolation from lymphocytes according to instructions. PCR samples were prepared by means of the set "AmpliSens-200-1".

The following primer set were used: to determine the C/T polymorphism in the DIO1 gene – forward – 5′-GAACTTGATGTGAAGGCTGGA-3′ and reverse – 5′-TAACCTCAGCTGGGAGTTGTTT-3′. Discrimination of DIO1 gene alleles was performed using the specific restriction enzyme Bcl I (Fermentas, USA).

Products of PCR were separated using electrophoresis in 3% agarose gel in the presence of tetraborate buffer, concentrated with ethidium bromide. Fragments were visualized by transilluminator in the presence of a marker of molecular mass 100–1000 bq (Fermentas, USA).

Pearson's χ^2-criterion was used to estimate the correspondence of the genotype frequencies in the study to theoretically expected distribution at Har-

dy-Weinberg's equilibrium. Odds ratio (OR) with determination of 95% confidence interval (CI) was calculated with the aim to establish the association of polymorphic variant of the gene with a pathological phenotype.

Blood samples taken after overnight fasting were analyzed for blood urea, serum creatinine. The eGFR was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) study formula [10]. Urine test was evaluated for microalbumin, creatinine level and albumin/creatinine ratio in biochemical laboratory. To assess thyroid hormone metabolism, TSH, free T_3 (fT_3), free T_4 (fT_4) were measured by ELISA method. TSH levels was determined using "Vector-Best" using standard set of reagents of the (Russia), fT_4 and fT_3 by "Granum" (Ukraine) set of reagents on the Uniplan enzyme-linked immunosorbent assay analyzer. Normal reference values for fT_3 are 4.0-8.6 pmol/l, for fT_4 – 10.3-24.5 pmol/l, for TSH – 0.3-4.0 mIU/l. Anthropometric indices were calculated: such as waist to hip (W/H) ratio and body mass index (BMI) by Quetelet, 1832.

All statistical computations were performed by means of the licensed software package Statistica for Windows 6.0, and Microsoft Excel 2016 table processor (Microsoft Corp., USA). For all parameters, in the groups of patients with CC, CT and TT genotypes and control group, the arithmetic mean (*M*), its dispersion and mean error *(m*) were calculated. All data corresponded to the normal distribution law. To determine the significance of differences between the results of research in groups of patients with CC, CT and TT genotypes and control group, the Student's coefficient (*t*) was calculated, after that the significance of the difference between the samples (*P*) and the confidence interval of the mean according to the Student distribution tables were determined. Valid values for $P < 0.05$ were considered statistically significant.

Results and Discussion

Disorders of distribution of genotype frequencies contributed by the reduction of CC genotype frequency was revealed in the group of enrolled patients comparing to the control group ($\chi^2 = 6.8$, $P < 0.05$), while there was no significant difference between the frequencies of CT and TT genotypes in the main and control groups ($\chi^2 = 2.4$, $P > 0.05$ and $\chi^2 = 1.2$, $P > 0.05$). Taking into account that the difference in genotypes frequencies occurs mainly due to a decrease in the number of patients homozygous for C allele, it can be assumed that the C allele has protective properties against deiodinase 1 activity reduction, that indicates the assotiation of C/T polymorphism in the *DIO1* gene with the development of thyroid hormone disturbances in the patients with DKD as compared to the control group.

Content of urea and creatinine in plasma, eGFR, as well as microalbumine and creatinine content in urine were significantly higher in patients with TT genotype than in group of patients with CC genotype and control group ($P < 0.05$). There was no significant intergroup difference of albumin/creatinine ratio according to the genotype for the *DIO1* gene ($P > 0.05$). Most of the indices of renal function were significantly worse in carriers of T-allele than in group of patients with CC genotype and control group ($P < 0.05$) (Table). Level of fT_4 was reliably higher in carriers of T-allele comparing to the group of patients homozygous for C allele and control group. fT_3 level was significantly lower in carriers of T-allele than in control group and group of patients with CC genotype ($P < 0.05$). There was no significant intergroup difference in the TSH level in blood plasma dependidng on C/T polymorphism in the *DIO1* gene.

Impaired biochemical parameters of renal function can be explained by suppression of peripheral metabolism of thyroid hormones on the background of diabetes mellitus with a decrease in the production of active T3 and the development of "low T_3" syndrome [4]. This process is triggered by the intensification of cytokines production on the background of type 2 DM as a result of the activation of cytokine expression by leptin and hyperglycemia. Cytokines, in turn, inhibit the activity of deiodnases, including D1, which is the most active [4]. Zheng Y. et al. Reported that low decreased of fT_3 and elevated TSH, even if they are still in normal ranges, are associated with increased risk of CKD [1]. Jingcheng Wu revealed negative correlation between the level of fT_3 and albumin/creatinine ratio and positive correlation between fT_3 and eGFR in patients with low normal T_3 level against the background on diabetic nephropathy [3].

It is known that the decrease in the level of thyroid hormones leads to disruption of micro- and macrocirculation due to stiffness of the vascular wall, inhibition of nitric oxide-stimulated vasodilation with the development of peripheral vasoconstriction [12,13]. In addition, it is known that a de-

Peculitaries of biochemical markers of renal function in patients with diabetic kidney disease according to C/T polymorphism in the DIO1 gene (M ± m)

Index, units of measurement	Genotypes of the GPX 1 gene, *n* = 102			Control group, *n* = 20
	CC, *n* = 19	CT, *n* = 69	TT, *n* = 14	
Blood urea, mmol/l	5.29 ± 0.34***	6.7 ± 0.62*	8.08 ± 1.19*	4.78 ± 0.65
Creatitinine, mmol/l	90.67 ± 8.59*/**/***	105.4 ± 20.61*	129.7 ± 15.37*	56.0 ± 7.78
Random microalbumine urine test, mg/dl	37.09 ± 7.26*/***	48.26 ± 7.87*	75.78 ± 16.36*	14.08 ± 5.27
Creatitinine in urine, mmol/l	8.0 ± 1.28***	10.26 ± 3.49	14.30 ± 2.58*	5.36 ± 0.64
Albumin/creatinine ratio, mg/mmol	70.41 ± 8.57	62.14 ± 8.36	57.38 ± 6.17*	86.35 ± 6.17
eGFR, ml/min	82.80 ± 7.14*/***	73.40 ± 8.63*	61.60 ± 5.22*	117.60 ± 12.43
TSH, mIU/l	2.93 ± 0.23	2.69 ± 0.16	2.65 ± 0.21	2,08 ± 0.15
fT_4, pmol/l	17.28 ± 2.35**/***	23.41 ± 1.74*	25.83 ± 2.06*	16.02 ± 1.45
fT_3, pmol/l	4.97 ± 0.15*/**/***	4.10 ± 0.04*	3.72 ± 0.07*	7.46 ± 0.17

n – number of observations; *the probability of changes in relation to control; **the probability of changes in relation to the group with CT-genotype; ***the probability of changes in relation to group with TT genotype

crease in thyroid hormone levels is accompanied by dyslipidemia and an increased risk of atherosclerotic vascular lesions, including the renovascular complications with the development of diabetic nephropathy [14-18]. Moreover, diabetic nephropathy can lead to changes in the content of thyroid hormones in the blood as a result of hypoproteinemia caused by severe proteinuria, because it is known that most of the thyroid hormones are bound to proteins [19]. So, these conditions can aggravate each other and need further investigation.

As follows, presence of the T allele in genotype is associated with violation of thyroid hormones metabolism with the development of nonthyroidal illness syndrome. Carriers of T allele with diabetic kidney disease had significantly worse biochemical indices of renal function, that indicates the dependence of these markers on *DIO1* polymorphism.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ ГЕНЕТИЧНИХ ФАКТОРІВ НА МЕТАБОЛІЗМ ТИРЕОЇДНИХ ГОРМОНІВ У ПАЦІЄНТІВ ІЗ ДІАБЕТИЧНОЮ ХВОРОБОЮ НИРОК

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У світі на цукровий діабет хворіє 1 із 11 дорослих. У 25% з них розвивається діабетична хвороба нирок. Тиреоїдні гормони беруть участь у регуляції практично всіх фізіологічних процесів в організмі, включаючи функцію нирок. Метою дослідження було вивчення залежності біохімічних маркерів функції нирок у пацієнтів із діабетичною хворобою нирок від C/T поліморфізму гена *DIO1*. Для оцінки залежності біохімічних маркерів функції нирок від C/T поліморфізму гена *DIO1* сформовано такі групи: 19 пацієнтів із генотипом CC, 69 – із CT та 14 – з генотипом TT. Вміст сечовини та креатиніну в плазмі, швидкість клубочкової фільтрації, а також вміст мікроальбуміну і креатиніну в сечі був значно вищим у пацієнтів з геноти-

пом ТТ, ніж у групі пацієнтів з генотипом СС та контрольною групою ($P < 0,05$). Дійшли висновку, що наявність Т-алелі в генотипі пов'язана з порушенням обміну тиреоїдних гормонів та розвитком синдрому нетиреоїдної патології. Носії Т-алелі з діабетичною хворобою нирок мали значно гірші біохімічні показники функції нирок, що свідчить про залежність цих маркерів від поліморфізму *DIO1*.

Ключові слова: С/Т поліморфізм гена *DIO1*, синдром нетиреоїдної патології, оцінка фукціонального стану щитоподібної залози, біохімічні показники функції нирок, діабетична хвороба нирок.

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