SHORT COMMUNICATIONS

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 dependence 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 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). 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.

Keywords: C/T polymorphism in the DIO1 gene, nonthyroidal 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 the 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 nonthyroidal illness syndrome (NTIS) or “low T₃ 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 triiodothyronine (T₃), high normal level of thyroxine (T₄), and inappropriately normal thyroid stimulating hormone (TSH) level in blood serum, but severe tissue hypothyroidism [4]. Deiodinase type 1 (D1) is an enzyme that is active in liver and kidneys and plays an important role in activation of prohormone T₄ to 5 times more active T₃ [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 \textit{DIO1} gene. We chose C/T polymorphism at position 785 in the \textit{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 \textit{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\textsubscript{3} (rT\textsubscript{3}) in plasma, an increase in rT\textsubscript{3}/T\textsubscript{4} ratio and a decrease in the T\textsubscript{3}/rT\textsubscript{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 \textit{DIO1} gene in patients with diabetic kidney disease.

\textbf{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 \textit{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 \textit{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 \textit{DIO1} gene – forward – 5’-GAACCTGATGTCAGGCTGGA-3’ and reverse – 5’-TAACCTCAGCCTGGAAGTTGTTT-3’. Discrimination of \textit{DIO1} gene alleles was performed using the specific restriction enzyme Bel 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 $\chi^2$-criterion was used to estimate the correspondence of the genotype frequencies in the study to theoretically expected distribution at Har-
**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 association 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 microalbumin 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 fT3 was reliably higher in carriers of T-allele comparing to the group of patients homoygous for C allele and control group. fT3 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 depending 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 T3” 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 deiodinases, including D1, which is the most active [4]. Zheng Y. et al. reported that low decreased of fT3 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 fT3 and albumin/creatinine ratio and positive correlation between fT3 and eGFR in patients with low normal T3 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-
increase 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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пом TT, ніж у групі пацієнтів з генотипом CC та контрольною групою (Р < 0,05). Дійшли висновки, що наявність Т-алелі в генотипі пов’язана з порушенням обміну тиреоїдних гормонів та розвитком синдрому нетиреоїдної патології. Но-сії 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 функціонального стану щитоподібної залози, біохімічні показники функції нирок, діабетична хвороба нирок.

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