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EFFECT OF ANGIOTENSIN-CONVERTING ENZYME GENE POLYMORPHISM AT THE RESULTS OF *LOSARTAN* USE IN THE PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Summary

The article presented is an original investigation where the effects of *losartan* in patients with hypertension and type 2 diabetes mellitus (T2DM) have been evaluated. The effect of therapy has been estimated with the help of gene ACE allelic variant. The dependence of *enalapril* antihypertensive action and nephroprotective action of ACE polymorphism has been revealed. The results obtained show a different effect on ACE allele variants of lipid metabolism in patients with hypertension and T2DM and complications in the form of hypertriglyceridemia in the patients with DD-genotype.

Key words: **type 2 diabetes mellitus, hypertension, I / D polymorphism of the gene ACE, pharmacogenetics, losartan.**

Thematic Justification. Diabetic nephropathy (DN) is the main cause of morbidity and mortality associated with diabetes mellitus (DM), but the clinical course and prognosis for the end - stage of chronic kidney disease (CKD) is highly variable for different patients [1]. DN has

several ultrastructural changes of renal tissue. Histologically at DN scoring of basal membrane is observed, as well as mesangial and tubular hypertrophy, proliferation of mesangial cells, glomerulosclerosis and tubulointerstitial fibrosis. Key clinical features that accompany these structural changes are albuminuria progressive increase, increased blood pressure (BP) and renal function decline with further development of uremia and CKD end-stage [2]. Combination of hemodynamic and metabolic mechanisms, growth factors, cytokines and genetic predisposition are triggers of these processes. At advanced stages, hemo- and non hemodynamic renal adaptive mechanisms are implemented in renal function disturbance, if renoprotective therapy has not been started. Activation of the renin-angiotensin-aldosterone system (RAAS) has a major role in the initiation of the pathological process and in the progression of diabetic kidney disease [3].

Angiotensin-converting enzyme (ACE) is found in epithelial and endothelial cells of various organs, including kidneys, heart muscle, lungs and vascular endothelial cells. Diabetes is one of the diseases which pathogenesis is conjugated with increasing concentrations of ACE. ACE gene is located on a human's chromosome 17 and contains a polymorphism in the form of insertions or deletions of DNA segments in the 16^{th} intron. The association between deletion polymorphism (I / D) of ACE gene and such conditions as coronary heart disease, hypertension, retinal artery disease and CKD is proved by numerous studies [4,5,6].

Polymorphism ACE and angiotensin II genes, genes of sodium-lithium co-transport violation, genetically determined unsulin resistance and hyperinsulinemia, loss of the negative charge by the basal membrane of renal glomeruli are used as genetic markers which determine predisposition to DN [7].

However, despite the large number of the studies that substantiate RAAS gene polymorphism with certain peculiarities of hypertension, risk of complications and progression of target organs disturbance, there was not found some association between the development of hypertensive nephropathy or CKD end-stage CKD and polymorphic variants of genes ACE and AT1R. Thus, today there are no clinical guidelines that consider appropriate therapeutic strategy based on genotypic variations of RAAS.

The objective: to analyze the dynamics of carbohydrate and lipid metabolism, average values of blood pressure and microalbuminuria aaat the background of the treatment with *losartan* as part of combination therapy in the patients with T2DM and hypertension, depending on the functional variant of insertion-deletion polymorphism of ACE gene.

Materials and methods. 79 patients with mean age 64.2 ± 4.2 y.o. among them 36 women and 43 men have been examined. The patients with T2DM with hypertension who have not been pre-treated with RAAS blockers were included into the study. Exclusion criteria was the level of glomerular filtration rate (GFR) <45 ml / min / 1.73 m^2, as it made difficult to

administer RAAS blockers and *metformin*. All the patients were examined according to the order of Ukrainian Ministry of Health Care №1118 dated 21.12.2012, №384 dated 24.05.2012 and standardized clinical protocols about rendering of primary and secondary health care to patients with T2DM and hypertension.

To determine the compensation of carbohydrate metabolism we studied blood glucose level by Hagedorn-Jensen's glucose oxidase test, in the fasting state and post-load. In order to evaluate the compensation of diabetes retrospectively we defined glycosylated hemoglobin (HbA1c) with a set of "Diabetes Test" (Phosphosorb, Russian Federation).

Microalbuminuria was diagnosed with immunoassay method with the use of «Micral-Test» (Boehringer Mannheim, Austria). GFR was calculated by formula CKD-EPI, according to the 2012 Kidney Disease Improving Outcomes Guidelines [8].

Genomic DNA was isolated from venous blood leukocytes using kits "DNA Sorb-B" (InterLabservis, Russian Federation). Polymorphic variants of ACE gene was assessed by polymerase chain reaction (PCR) using sets of reagents for amplification «SNP-Express" (Russian Federation). PCR was performed on amplifier BIO-RAD (USA). 100-150 ng of DNA was used for amplification. The initial denaturation was made at 95 ° C for 10 min. PCR was made for 40 cycles: denaturation at 95°C was made 30 sec, annealing at 64°C - 30 sec., elongation - at 72°C for 30 sec and ultimate elongation - 3 minutes at 72°C. Separation of the amplification products was performed in horizontal 2% agarose gel prepared on one-use TBE.

All the patients restrict carbohydrate intake from food and received a balanced diet at the table № 9 by Pevzner. The patients got standard treatment of diabetes with *per os* hypoglycemic agents (mostly metformin, or a combination of metformin with inhibitor of DPP or biguanides). As antihypertensive and nephroprotective therapy all the patients gota daily dose of *losartan* 10 mg in two divided doses. Dyslipidemia patients were treated with a daily dose of *rosuvastatin* (10-20 mg), in the presence of severe hypertriglyceridemia *fenofibrate* was administered at a dose of 145 mg per day. Clinical examination was performed in two stages - before the treatment and in 12 weeks after the combined treatment beginning.

Statistical analysis of the results obtained was performed using the software Statistica 10.0 (StatSoft, USA). Signs distribution normality was checked with the Shapiro-Wilkie's criterion, statistical reliability of differences between groups was assessed with analysis of variance ANOVA. Indexes of microalbuminuria and GFR, with taken into account the distribution of parameters other than normal were analyzed by nonparametric methods.

Results and discussion. Groups of the patients obtained by the results of genotyping were matching by age and sex. The number of monozygotic for deletion variant of ACE genotype patients was significantly higher in the group under study and higher in comparison to

other studies with the representatives of Ukrainian and other Eastern European populations as an object [9,10]. Existing realized genetic risk in the group of older people with manifesting hypertension and comorbidities may explain such a high prevalence of potentially alternating allelic variant.

Table 1.

Distribution of the patients in the groups by the variants of ACE gene polymorphism

ACE genotype	II	ID	DD
Number of the patients, abs., %	14 (17.72%)	13(16.46%)	52(65.82%)
Women, abs., %	7 (50%)	5(38.46%)	24(46.15%)
Male, abs.%	7(50%)	8(61.54%)	28(53.85%)
The mean age of patients, y.o.	64.2±3.0	61.7±4.2	64.5±4.8

In all parameters the groups of the patients according to the detected genotypic variants were comparable before the treatment, except for distributions of concentrations of triglycerides and LDL cholesterol (Table 2). Further *post hoc* analysis (including Bonferoni's amendments at multiple comparison of significance test adopted p = 0,01) before treatment showed statistically significant differences in the concentration of triglycerides in patients with genotype II and DD (p_{II-ID} = 0,35; p_{ID-DD} = 0 14; p_{II-DD} = 0,006) with moderate prevalence of this indicator in II genotype patients. Concentrations of LDL cholesterol with taking into account Bonferoni's correction had no statistically significant differences (p_{II-ID} = 0,08; p_{ID-DD} = 0,73; p_{II-DD} = 0,019).

After the treatment, there were some certain differences in the distribution of the parameters under study in the groups. It indicates that the effect of pharmacotherapy was different and depended on the genotype (Table 2). As follows from the analysis of the parameters under study distribution in the groups before and after the treatment statistically significant difference in the mean value of office SBP in all groups were present, while the decline rate of office DBP was statistically significant only in genotype II patients. These observations may indicate about significant nephrogenic component of diastolic hypertension in the group of D-allele carriers and thus suboptimal antihypertensive effect in this case.

On the data of carbohydrate metabolism, statistically significant changes due to the treatment were observed in the groups of I-allele carriers (p = 0.001 for genotype II and p = 0.038 for genotype ID). The best result in terms of postprandial glycemia was achieved in the patients with genotype II (p = 0.038).

The best results by the levels of microalbuminuria in the form of a significant reduction of the daily excretion of albumin with urine was achieved in the patients – carriers of I -allele (p = 0.024 for genotype II and p = 0.005 for genotype ID).

Lipid profile in response to combined therapy experienced significant changes in all the patients, but to achieve target values of total cholesterol was possible only in heterozygous genotype group. Value of LDL as a result of the treatment significantly decreased in genotype II patients, but the target levels (<1.8 mmol / L) has been reached in none of the groups (Global Guidelines on Type 2 Diabetes Mellitus, International Diabetes Federation, 2012). Reduced serum TG levels was achieved in the groups of I-allele ACE gene carriers. Triglyceride levels in patients with homozygous deletion variant of ACE genotype underwent adverse changes. It should be noted that most of the patients did not need fibrates administration, but in 12 weeks high levels of serum triglycerides (> 2.3 mmol / l) were found in 16 (30.76%) patients with DD-genotype.

Table 2.

Clinical parameters of the patients before the treatment and in 12 weeks of *losartan* intake

Parameter		Before treatment	After treatment	p_2
1		2	3	4
Mean values of office SBP, mm Hg	II	144.6 ± 8.2	135.7 ± 5.8	0.001
	ID	146.9 ± 5.8	136.7 ± 7.2	0.002
	DD	145.8 ± 8.9	143.1 ± 8.8	0.006
	p_1	0.59	0.003	-
Mean values of office DBP, mm Hg	II	91.1 ± 4.9	83.2 ± 3.7	0.002
	ID	85.8 ± 7.0	82.9 ± 4.0	0.123
	DD	88.3 ± 6.3	88.1 ± 6.6	0.749
	p_1	0.13	0.002	-
Fasting blood glucose, mmol / L	II	6.1 ± 1.1	5.6 ± 0.8	0.148
	ID	5.7 ± 1.7	5.7 ± 0.8	0.859
	DD	5.8 ± 1.5	6.6 ± 0.7	0.918
	p_1	0.13	0.77	-
Postprandial glycemia, mmol / L	II	7.0 ± 1.2	6.6 ± 0.7	0.038
	ID	7.2 ± 1.4	7.1 ± 0.6	0.937
	DD	7.7 ± 1.4	7.7 ± 1.0	0.924
	P_1	0.52	0.001	-
HbA1c,%	II	7.0 ± 1.2	6.5 ± 1.1	0.001
	ID	6.6 ± 1.5	5.2 ± 0.6	0.038
	DD	6.6 ± 1.4	5.9 ± 0.7	0.187
	p_1	0.46	0.001	-

EGFR, mL / min / 1.73 m^2	II	78.0 (66.0; 88.0)	76.0(64.0;80.0)	0.140
	ID	75.5 (54.0;81.5)	76.0(53.0;83.0)	0.695
	DD	72.5(56.0;84.5)	73.5(59.5;82.5)	0.145
	p_1	0.28	0.89	-
Creatinine, μmol / l	II	89.6± 11.1	90.6 ± 16.7	0.937
	ID	89.6±13.0	86.5 ± 12.1	0.556
	DD	97.5±22.1	96.8 ± 21.0	0.838
	p_1	0.38	0.15	-
Microalbuminuria, mg /	II	150.0(85.0;360.0)	68.0 (56.0;105.0)	0.024
	ID	145.0(135.0;300.0)	67.5 (55.0;85.0)	0.005
	DD	145.0(109.0;250.0)	146.5(91.0;183.0)	0.988
	p_1	0.86	0.001	-
Total cholesterol, mmol/L	II	5.8 ± 0.5	5.1 ± 0.5	0.003
	ID	5.6 ± 0.4	4.8 ± 0.4	0.003
	DD	5.7 ± 0.6	5.5 ± 0.7	0.029
	p_1	0.43	0.005	-
HDL cholesterol, mmol/L	II	1.1 ± 0.4	1.2 ± 0.3	0.397
	ID	1.1 ± 0.2	1.2 ± 0.3	0.415
	DD	1.2 ± 0.3	1.2 ± 0.3	0.385
	p_1	0.85	0.89	-

1		2	3	4
LDL cholesterol, mmol/L	II	4.1 ± 0.5	3.7 ±0.4	0.011
	ID	3.8 ±0.3	3.6 ± 0.3	0.328
	DD	3.8 ±0.3	3.7 ± 0.4	0.365
	p_1	0.05	0.69	-
TG mmol/l	II	2.6 ± 0.5	2.0 ± 0.4	0.019
	ID	2.4 ±0.8	1.6 ± 0.6	0.012
	DD	2.1 ± 0.7	2.5 ± 0.7	0.006
	p_1	0.01	0.001	-

p_1 - the criterion of significance when testing the hypotheses about the lack of statistically significant differences between three independent groups of patients. Calculated by Kraskel-Wallis's criterion.
p_2 - the criterion of significance when testing the hypothesis about the lack of difference between two dependent groups (before and after the treatment with losartan). Calculated by Wilcoxon's criterion.

Conclusions. Thus, variant of insertion-deletion polymorphism of ACE has a significant impact on the antihypertensive and nephroprotective effects of *losartan.* The most significant predictor of relative optimal **antyproteyinurichnoyi** action of *losartan* is carrier state of ACE I-allele, patients with II and ID variants of genotype ACE had the most significant decrease in microalbuminuria indexes. Carrier state of ACE gene D-allele is a factor of not only potentially inadequate response to antihypertensive and nephroprotective therapy, but also it may be a reason of poorer compensation of carbohydrate and lipid metabolism. The question of rational

pharmacotherapy in such cases should be decided individually by rational selection of antihypertensive agents combination, for example, by adding to RAAS calcium channel blockers or low doses of diuretics.

An important step towards understanding of predictive and pharmacological significance of ACE gene polymorphism are further large-scale studies with the long-term observation. It will help to fix the frequency of cardiovascular and renal events in patients with T2DM at the background of RAAS blockers treatment.

Determination of genotype of ACE gene in patients with type 2 diabetes and hypertension is effective step to personalized appointment of antihypertensive therapy RAAS blockers and other antihypertensive drugs classes to achieve target blood pressure levels.

References:

1. P.K. Jacobsen. Preventing and stage renal disease in diabetic patients - genetic aspect. Journal of the Renin-Angiotensin-Aldosterone System 2005; 6: 1-14.

2. P. Ruggenenti, P.Cravedi, G, Remuzzi. The RAAS in the pathogenesis and treatment of diabetic nephropathy. Nat. Rev. nephrol. 6, 319-330 (2010).

3. G. Braliou, A.G. Grigoriadou, P.I. Kontou, P.G. Bagos. The role of genetic polymorphisms of the reninb-angiotensin system in trenal diseases: a meta-analysis. Computational ands Structural Biotechnology Journal 10 (2014) 1-7.

4. M. Zivko et al. Impact of Angiotensin-Converting Enzyme gene polymorphism on proteinuria and arterial hypertension. Coll. Antropol. 37 (2013) 3: 765-770. (Rus.)

5. H.E.Roytberh, Zh.V.Dorosh, Ye.V.Aksonov, T.I.Ushakova. Effect of polymorphism of angiotensin-converting enzyme on the occurrence of insulin resistance syndrome. Clinician. - №2,2013 - P. 14-17 (Rus.).

6. Haque S.F. et al. ACE insertion / deletion gene polymorphism determines efficacy of telmisartan: an ARB in type 2 diabetic nephropathy. Journal of cell and tissue research. 2011. - Vol.11 (1): 2595-2600.

7. Cooper Worobey C., Fisher NDL, Cox D, Forman JP, Curhan GC. Genetic polymorphisms and the risk of accelerated renal function decline in women. PLoS ONE 4 (3): e4787. doi: 10.1371 / journal.pone.0004787.

8. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International Supplement. - 2013. - №3. - R.1-150.

9. AE Dubenko, VV Hvysyuk, SL Kostyukovsky, T.V.Danko. Microalbuminuria and gene polymorphism of angiotensin-converting enzyme inhibitors in patients with acute stroke. Problems of Continuing Education and Science of Ukraine. - №1, 2014. - P.38-41 (Ukr.).