

**THE ASSOCIATION OF LIPID SPECTRUM WITH THE *ESR1* GENE
POLYMORPHISM IN POSTMENOPAUSAL WOMEN WITH
ARTERIAL HYPERTENSION AND TYPE 2 DIABETES MELLITUS**

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Abstract.

The purpose of the research was to determine the relationship between the level of serum lipids and polymorphism of the estrogen alpha receptor gene (*ESR1*) in postmenopausal women with hypertension and type 2 diabetes mellitus.

Materials and methods. A total of 137 caucasian women residing in the territory of Kharkiv city and Kharkiv region were examined. Women were receiving an inpatient treatment at endocrinology and cardiology units of "Regional Clinical Hospital - Center for emergency medical care and disaster medicine" in Kharkiv. Patients were divided into the following groups: 1st - patients with type 2 diabetes mellitus (n = 30), 2nd - patients with arterial hypertension, grade 2-3 (n = 34), 3rd – comorbid hypertension of grade 2-3 and type 2 diabetes (n = 42). The control group included 31 women in early natural menopause, which had normal blood pressure, glycated hemoglobin (HbA1c) level below 6% and absent endocrine diseases. The age of examined patients varied between 45 and 60 years.

The following indexes were measured to study the lipid profile: triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Using polymerase chain reaction (PCR) was determined *ESR1* gene T397C polymorphism in DNA samples. Identification of polymorphisms led to three types of conclusions: normal homozygotes (TT), heterozygotes (TC) and homozygous mutation (CC).

Results. Our research has shown that the presence of diabetes might contribute to the worsening of the blood lipid profile in postmenopausal patients. The changes of blood lipid profile were associated with predominance of heterozygous TC genotypes of ESR1 gene in postmenopausal patients. Postmenopausal women either with hypertension alone or with comorbid course of hypertension and type 2 diabetes are characterized by significant predominance of heterozygous TC polymorphism T397C of ESR1 gene. Also, these patients have more pronounced changes of the lipid profile.

Conclusions. According to the results of our study, we proposed a marker for targeted prevention of cardiovascular disease in postmenopausal women, which is the identification of heterozygous TC genotype T397C polymorphism of ESR1 gene against the background of marked lipid profile changes.

Keywords: gene polymorphism, lipid profile, diabetes mellitus, arterial hypertension, postmenopause.

Introduction.

In a modern world the development and use of high technology and communications leads to the well-being of humanity and increase of life expectancy. Therefore, the growing proportion of old people in the human population is not a surprise. In regard to modern women, they spend almost a third of their lives having a sex hormone deficiency [15]. Women in menopause have increased risk of cardiovascular disease (CVD), severity of which can be influenced by the presence of type 2 diabetes mellitus (DM) [2]. The study «The Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe» had shown that women with comorbid diabetes and arterial hypertension (AH) have two-fold higher risk of death than men. [4]

One of the greatest achievements of modern medical science is the insight into the molecular genetic nature of diseases [3,11]. Determination of high-risk groups for the development of the disease on the basis of a specific genotype is of great practical importance. The advantage of molecular genetic research is that it gives an opportunity for presymptomatic disease diagnosis. The determination of a disease association with genetic marker suggests a presence of predisposition to the development of

pathological process, predicts the progression of the disease and its possible severity, helps to make an early diagnosis and to perform a targeted prevention.

The estrogen alpha receptor gene ESR1 has been considered in our research. This gene possesses several polymorphisms, which have been already detected and studied. In our study we examined the PvuII-polymorphism (T397C) in the following categories of postmenopausal women: healthy volunteers, patients with hypertension, patients with type 2 diabetes, and women with comorbid course of hypertension and diabetes. This type of polymorphism is characterized by the substitution of nucleotide thymine (T) to cytosine (C) in the non-coding region of a gene that affects the PvuII restriction site. The prevalence rate of the mutant variant of ESR1 gene in European population is 42-45%. The mutation is inherited in an autosomal dominant manner and affects both men and women with the same frequency. It is enough to inherit one mutant version of the gene from one parent for the development of the disease. The probability of occurrence of the disease in children is 50%. The function of the gene is encoding of receptor of estrogen alpha hormone [10, 7].

According to the survey of international scientific publications, there are articles devoted to the study of the association of PvuII-polymorphism of ESR1 gene with blood lipid profile in various pathological conditions depending on gender [9, 13, 6].

The reports of different research groups confirm the fact that changes of blood lipid profile in postmenopausal women can contribute to the increase of the cardiovascular risk [5, 10].

The purpose of the research was to determine the relationship between the level of serum lipids and polymorphism of the estrogen alpha receptor gene (ESR1) in postmenopausal women with hypertension and type 2 diabetes mellitus.

Methods.

A total of 137 caucasian women residing in the territory of Kharkiv city and Kharkiv region were examined. Women were receiving an inpatient treatment at endocrinology and cardiology units of "Regional Clinical Hospital - Center for emergency medical care and disaster medicine" in Kharkiv. Before inclusion in this study all patients were acquainted with goals, objectives, stages and medical procedures of the research according to the requirements of the Declaration of Helsinki

and the current legislation of Ukraine on the issues of ethical principles for medical research. All examined women have signed an informed consent to participate in the study. Patients were in early natural menopause and didn't have any serious associated disease like nephropathy, acute cardiovascular disease, cerebrovascular accidents, acute inflammation and exacerbation of chronic inflammatory diseases, oncological diseases, toxic and viral hepatitis, allergic reactions, bad habits (tobacco and alcohol addiction).

Patients were divided into the following groups: 1st - patients with type 2 diabetes mellitus (n = 30), 2nd - patients with arterial hypertension, grade 2-3 (n = 34), 3rd – comorbid hypertension of grade 2-3 and type 2 diabetes (n = 42). The control group included 31 women in early natural menopause, which had normal blood pressure, glycated hemoglobin (HbA1c) level below 6% and absent endocrine diseases.

The description of examined patients, according to research groups, is given in Table 1 and 2. At the time of the study, patients with type 2 diabetes were in a stage of subcompensation, state of suboptimal glycemic control (HbA1c <7.5%). Women with hypertension had a level of HbA1c <6%. Groups were comparable in terms of general age, age of menopause onset, duration of postmenopausal period, body mass index (BMI). Patients received no hormone or lipid-lowering therapy prior to admission and enrollment in the study. During treatment in the hospital patients received therapy according to current clinical guidelines.

The age of examined patients varied between 45 and 60 years. Data on the age of natural menopause onset and duration of postmenopausal period were collected from the patients' anamnesis. The duration of postmenopausal period varied from 2 to 6 years. The level of follicle stimulating hormone (FSH) was determined in blood serum of all patients by ELISA method using a kit of reagents for the enzyme immunoassay produced by "Hema" (Russian Federation). Menopause was diagnosed when FSH level was greater than 25 mIU/ml. Age-related parameters of patients depending on the study group are shown in Table 1.

Table 1

Age-related indexes according to study groups, $M \pm m$

Index	Groups			
	1 st (patients with DM)	2 nd (patients with AH)	3 rd (patients with DM and AH)	Control group
Age, years	51.43±0.97	53.36±0.82	52.05±0.74	53.77±0.81
Onset of the natural menopause, years	47.53±1.02	49.26±0.91	47.79±0.73	49.23±0.86
Duration of postmenopausal period, years	3.90±0.25	4.11±0.24	4.26±0.21	4.54±0.22

Body height and weight were measured in fasting patients. BMI was calculated according to Quetelet's formula:

$$\text{BMI} = m \text{ (kg)} / h \text{ (m)}^2 ,$$

m - the body weight in kilograms

h - height in meters.

The received anthropometric data, as well as the BMI, were interpreted in accordance with the WHO recommendations (Table 2).

Table 2

Anthropometric data of patients in studied groups, $M \pm m$

Index	Группы			
	1 st (patients with DM)	2 nd (patients with AH)	3 rd (patients with DM and AH)	Control group
Height, m	1.67±0.015	1.67±0.016	1.65±0.011	1.65±0.016
Weight, kg	68.57±1.53	69.09±1.80	68.88±1.52	68.77±1.45
BMI, kg/m ²	23.67±0.58	24.18±0.64	24.50±0.52	24.65±0.60

The diagnosis of type 2 diabetes mellitus was set in accordance with the classification of glycemia disorders (WHO, 2006 and the order of the Ministry of Health of Ukraine № 1118 from 22.12.2012) [14].

Verification of AH diagnosis was performed according to the recommendations of the European Society of Hypertension (the ESH) and the European Society of Cardiology (the ESC) (2013) [16], the recommendations of Ukrainian Association of Cardiology (2008) [12] and the Order of the Ministry of Health of Ukraine № 384 from 24.05.2012. Arterial hypertension in the studied patients was caused by essential hypertension (EH), stage II, grade 2-3. The stage of EH was set according to classification, developed by WHO experts (1963-1993), adopted in Ukraine in 1992 (the Order of Ministry of Health of Ukraine № 206 from 30.12.1992 and recommended for further use).

The following indexes were measured to study the lipid profile: triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). The measurement was performed by an enzymatic method using a biochemistry analyzer Stat fax 1904 plus and test kits of Bio Merieux Company (France). Sentinel test system (Italy) was used to determine the content of TG.

Genomic DNA was isolated from leukocytes of venous blood using NeoPrep100 DNA reagent kit (set by NEOGENE Company (Ukraine)). Polymerase chain reaction (PCR), aimed to isolate a T397C polymorphism of ESR1 gene, was performed on isolated DNA samples by agarose gel electrophoresis using a set of reagents produced by NEOGENE company (Ukraine). Identification of polymorphism was performed by allele-specific PCR (AS-PCR). The results of analysis led to three types of findings: normal homozygote (TT), heterozygote (TC) and mutational homozygote (CC).

The research results were statistically treated using patient cards, adapted for Microsoft Excel program processing. The obtained data were evaluated by the average value (M) and standard deviation (m). The significance of indexes difference was determined by Student's t-test. Difference was considered significant when the value of t-test corresponded to 95% ($p < 0.05$). [8].

Results and discussion.

Our research has shown that the indexes of lipid profile in studied groups were significantly higher in comparison to healthy volunteers ($P < 0.05$) (Table. 3). At the same time, we have observed that the homozygous TT genotype significantly ($p < 0.05$) prevailed in healthy volunteers compared to groups of studied patients (Table. 4). When considering the lipid profile in patients with diabetes (group 1), the studied indexes were significantly lower than the ones in patients of group 2 (patients with hypertension) and patients of group 3 (patients with hypertension and diabetes). In this case, HDL-C component was the only exception, because it was significantly lower in all groups of patients in comparison with the control group ($p < 0.05$), except of the group 1 (patients with DM), in which this statistical parameter wasn't found significant in comparison with healthy volunteers ($p > 0.05$). An interesting fact is that the value of HDL-C significantly differed between the groups of patients and was higher in the first group (diabetic patients) compared to second (hypertension patients) and 3rd (hypertension and diabetes patients) groups. A comparison of the second (hypertension patients) and third group (patients with AH and DM) showed that HDL-C was significantly higher in patients of group 2 (Table 4), which may indicate the influence of lipid profile changes on the development of hypertension. The presence of diabetes might contribute to the worsening of the mentioned above indicators.

The changes of blood lipid profile were associated with predominance of different genotypes of ESR1 gene in postmenopausal patients. Thus, the presence of a heterozygous TC genotype significantly prevailed in group 2 (hypertension patients) and group 3 (patients with AH and DM), $p < 0.05$. Patients of group 1 demonstrated an insignificant difference between homozygous CC and heterozygous TC genotypes, however, these genotypes were found significantly more frequently than homozygous TT genotype (Table 4).

Table 3

Indexes of lipid profile, $M \pm m$

Groups	n	Indexes of lipid profile			
		TG	TC	HDL-C	LDL-C

		(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)
1 st (patients with DM)	30	1,92±0,14*•	5,24±0,19*•	1,28±0,03•	3,90±0,19*•
2 nd (patients with AH)	34	2,47±0,13*#	5,95±0,18*#	1,10±0,03*#	4,77±0,19*#
3 rd (patients with DM and AH)	42	3,10±0,11*°	6,92±0,13*°	0,87±0,02*°	5,63±0,11*°
Control group	31	1,33±0,12	4,37±0,12	1,34±0,03	2,98±0,11

Note:

* Difference is significant ($p < 0.05$) when compared to healthy volunteers (control group);

• Significant difference between groups 1 and 2 ($p < 0.05$);

° Significant difference between groups 1 and 3 ($p < 0.05$);

Significant difference between groups 2 and 3 ($p < 0.05$).

Table 4

*Distribution of polymorphism T397S genotypes of ESR1 gene
in postmenopausal women*

Groups	n	Genotypes		
		TT	TC	CC
1 st (patients with DM)	30	10.00±5.48% (n=3)	56.67±9.05% (n=17)	33.33±8.61% (n=10)
2 nd (patients with AH)	34	8.82±4.86% (n=3)	67.65±8.02% (n=23) [°]	23.53±7.27% (n=8)
3 rd (patients with DM and AH)	42	11.90±5.00% (n=5)	61.90±7.49% (n=26) [°]	26.19±6.78% (n=11)

Control group	31	70.97±8.15% (n=22)*	12.90±6.02% (n=4)	16.13±6.61% (n=5)
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Note:

* - Difference is significant when comparing ($p < 0,05$) homozygous TT genotype in the group;

Ò - Difference is significant when comparing ($p < 0,05$) heterozygous TC genotype in the group.

Better understanding of the processes that develop in the women body during menopause will help to develop new approaches to treatment and will contribute to the improvement of medical aid for women of this age period. Determination of genotypes will enable scientists to highlight distinct groups of patients who are more prone to certain disease and its complications. Primary prevention of hypertension in postmenopausal women, which would include the identification of genetic markers of the disease, should be considered as promising approach to improvement of women health.

Conclusions.

Our study has demonstrated that postmenopausal women either with hypertension alone or with comorbid course of hypertension and type 2 diabetes are characterized by significant predominance of heterozygous TC polymorphism T397C of ESR1 gene. Also, these patients tend to have more pronounced changes of the lipid profile. Thus, according to the results of our study, we proposed a marker for targeted prevention of cardiovascular disease in postmenopausal women, which is the identification of heterozygous TC genotype T397C polymorphism of ESR1 gene against the background of marked lipid profile changes.

Determination of genetic susceptibility to disorders of lipid profile in postmenopausal women would help to develop an individual prevention measures against exposure to risk factors and, thus, would allow to prevent, delay and reduce the severity of the disease.

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АССОЦИАЦИЯ ЛИПИДНОГО СПЕКТРА С ПОЛИМОРФИЗМОМ ГЕНА *ESR1* У ЖЕНЩИН В ПОСТМЕНОПАУЗЕ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ И САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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Реферат.

Целью исследования было определить взаимосвязь между уровнем липидов в сыворотке крови и полиморфизмом гена рецептора эстрогена альфа (*ESR1*) у женщин в постменопаузе с артериальной гипертензией и сахарным диабетом 2 типа.

Материалы и методы. Обследовано 137 женщин, проживающих на территории г. Харькова и Харьковской области. Женщины получали стационарное лечение в эндокринологии и кардиологии "Областной клинической больницы - Центра экстренной медицинской помощи и медицины катастроф" г. Харькова. Пациентки были разделены на группы: 1-я - пациентки с сахарным диабетом 2 типа (n=30), 2-я - пациентки с артериальной гипертензией 2-3 степени (n=34), 3-я - гипертензия 2-3 степени и сахарный диабет 2 типа (n=42). В контрольную группу вошла 31 женщина в начале естественной менопаузы, которая имела нормальное кровяное давление, уровень гликированного гемоглобина (HbA1c) ниже 6% и без эндокринных заболеваний. Возраст обследованных колебался от 45 до 60 лет.

Следующие показатели были измерены для изучения липидного профиля: триглицериды (ТГ), общего холестерина (ТС), холестерина липопротеинов высокой плотности (ЛПВП) и холестерина липопротеинов низкой плотности (ХС-ЛПНП). С помощью полимеразной цепной реакции (ПЦР) определяли Т397С полиморфизм гена *ESR1* в образцах ДНК. Идентификация полиморфизма привела к трем типам выводов: нормальный гомозиготы (ТТ), гетерозиготы (ТС) и мутационный гомозиготных (СС).

Результаты. Наше исследование показало, что наличие диабета может способствовать ухудшению липидного профиля крови в постклимактерическом периоде изменения пациенток. Липидный профиль крови был связан с преобладанием гетерозиготного ТС генотипа гена *ESR1* у женщин в постменопаузе. Женщины с артериальной гипертензией или с сопутствующим сахарным диабетом 2 типа характеризуются значительным преобладанием гетерозиготного ТС полиморфизм Т397С гена *ESR1*. Кроме того, эти пациенты имеют более выраженные изменения липидного профиля.

Выводы. По результатам нашего исследования, мы предложили маркер для

целенаправленной профилактики сердечно - сосудистых заболеваний у женщин в постменопаузе: выявление гетерозиготного генотипа TC T397C полиморфизма гена *ESR1* на фоне изменений липидного профиля.

Ключевые слова: полиморфизм гена, липидный профиль, сахарный диабет, артериальная гипертензия, постменопауза.

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