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EVALUATION OF METABOLIC DISORDERS AND AGING RATES DEPENDING ON *SIRT1* POLYMORPHISM IN PATIENTS WITH ARTERIAL HYPERTENSION AND SUBCLINICAL HYPOTHYROIDISM

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Ключові слова: *артеріальна гіпертензія, субклінічний гіпотиреоз, старіння, метаболічні захворювання, SIRT1*
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Abstract. Evaluation of metabolic disorders and aging rates depending on *SIRT1* polymorphism in patients with arterial hypertension and subclinical hypothyroidism. Radchenko A.O., Kolesnikova O.V. *There is an increase in the frequency and severity of metabolic disorders in patients with arterial hypertension (AH) in combination with subclinical hypothyroidism (SH), that is accompanied by accelerated aging rates, but the research findings on the aging rates in this category of patients are extremely few in number. Therefore, the aim of our study was to assess metabolic disorders and the aging rates depending on the SIRT1 rs7069102 polymorphism in patients with AH and SH. A total of 132 patients with a median age of 47.6 years were included in the study and divided into 3 groups: a control group (n=30), a group of patients with AH without SH (n=49) and patients with AH in combination with SH (n=53). Anthropometric parameters, biochemical parameters, pro-inflammatory and oxidative states were evaluated in all patients. The aging rates were assessed using two different methods. The frequencies of SIRT1 rs7069102 genotypes carriers in the study sample of patients were 8% for the CC genotype, 51% for the CG genotype and 41% for the GG genotype. There was a significant difference in the incidence of CC homozygosity and carriers of the G allele between groups of patients with AH depending on the SH presence (p<0.001). We showed that carriers of the G allele and GG genotype of the SIRT1 gene (rs7069102) polymorphic marker with AH and SH had significantly higher (p<0.05) insulin resistance, higher levels of low-density lipoprotein cholesterol, alkaline phosphatase, C-reactive protein and lower glomerular filtration rate, which negatively affected the aging processes in this category of patients. In addition, patients with AH had a marked effect of carrying the G allele on the lipid profile and biological age of patients. Therefore, timely detection of a polymorphic variant of the SIRT1 gene may be effective in premature aging prevention in patients with AH and SH.*

Реферат. Оцінка метаболічних порушень та темпів старіння залежно від поліморфізму *SIRT1* у пацієнтів з артеріальною гіпертензією та субклінічним гіпотиреозом. Радченко А.О., Колеснікова О.В. *У пацієнтів з артеріальною гіпертензією (АГ) у поєднанні із субклінічним гіпотиреозом (СГ) спостерігається збільшення частоти і вираженості метаболічних порушень, що супроводжується прискоренням темпів старіння, проте результати досліджень швидкості старіння в цієї категорії пацієнтів вкрай нечисленні. Тому метою нашого дослідження стала оцінка метаболічних порушень та темпів старіння залежно від поліморфізму rs7069102 SIRT1 у пацієнтів з АГ та СГ. Всього в дослідження було включено 132 пацієнти, медіана середнього віку дорівнювала 47,6 року, які були розподілені на 3 групи: групу контролю (n=30), групу пацієнтів з АГ без СГ (n=49) та пацієнтів з АГ у поєднанні з СГ (n=53). Усім пацієнтам було оцінено антропометричні показники, біохімічні показники, прозапальний та оксидативний стани. Темпи старіння пацієнтів оцінювали за двома різними методами. Частота зустріваності носіїв різних генотипів rs7069102 SIRT1 у досліджуваній вибірці становила 8% для СС генотипу, 51% для CG генотипу та 41% для GG генотипу. Спостерігалась достовірна різниця в частоті зустріваності гомозигот С/С та носіїв алеля G між групами пацієнтів з АГ залежно від наявності СГ (p<0,001). Нами було показано, що носії алеля G та генотипу GG поліморфного маркера гена SIRT1 (rs7069102) з АГ та СГ мали достовірно вищі (p<0,05) показники інсулінорезистентності, вищі рівні холестерину ліпопротеїнів низької щільності, лужної фосфатази, С-реактивного білка та нижчу швидкість клубочкової фільтрації, що негативно впливало на процеси старіння в цієї категорії хворих. Окрім того, у пацієнтів з АГ був помітний вплив носійства алеля G на показники ліпідного профілю та біологічний вік хворих. Тому своєчасне визначення поліморфного варіанту гена SIRT1 може бути ефективним для профілактики передчасного старіння в пацієнтів з АГ та СГ.*

The study of the aging process, its rate, mechanisms and markers in young and middle aged people in order to increase life expectancy among older adults is extremely important. The presence of cardiovascular disease (CVD) is the main cause of premature aging in most cases, because both the development of CVD and aging have common pathogenetic mechanisms, the key of which are inflammation of low grades and oxidative stress. In turn, the pro-inflammatory state and the violation of redox balance lead to the further development of metabolic disorders and, as a consequence, the progression of CVD and increased aging rates [1].

Arterial hypertension (AH) is one of the most com mon CVD worldwide and and most often develops on the background of subclinical hypothyroidism (SH), which is not diagnosed timely and leads to profound metabolic disorders. Patients with AH and SH have similar common cardiovascular risk factors [2]. A significant role in the pathogenesis of these diseases is played by genetic factors, which include polymorphism rs7069102 *SIRT1* (sirtuin 1), that regulates intracellular processes, affects aging, participates in stress and inflammatory reactions, inhibiting the expression of tumor necrosis factor alpha (TNF-α) etc., however, data on the role of this exact polymorphism in patients with chronic cardiometabolic pathologies are limited [3, 4]. Therefore, the study of *SIRT1* polymorphism in patients with a combined course of AH and SH may be a source of future treatment and prevention measures development.

Our aim was to evaluate metabolic disorders and the rate of aging depending on the *SIRT1* rs7069102 polymorphism in patients with AH and SH.

MATERIALS AND METHODS OF RESEARCH

The study included 132 patients with a median age of 47.6 [39.5;54.7] years, among whom women accounted for 53% (n=70), who underwent outpatient and inpatient treatment at the L.T. Mala Therapy National Institute of the National Academy of Medical Sciences of Ukraine from 2019 to 2021. The study included patients with stage I-II grade 1-2 AH, who were divided into 2 groups depending on thyroid function: group 1 (comparison) – 49 patients with signs of autoimmune thyroiditis (AIT) without SH; group 2 (main) – 53 patients with SH and signs of AIT. The age-and gender-matched control group consisted of 30 healthy volunteers. AH was diagnosed in accordance with the 2018 European Society of Cardiology/European Society of Hypertension Guidelines for the management of AH. The diagnosis of AIT was verified by ultrasound examination of the thyroid gland, determination of antibodies to thyroperoxidase and thyroid function in all patients. The diagnosis of SH was made in accordance with the recommendations of the European Thyroid Association (2013).

Evaluation of anthropometric parameters, lipid, carbohydrate profile, kidney and liver function was performed according to the generally accepted method. Additionally, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and glomerular filtration rate (GFR) were calculated. The proinflammatory state was assessed by measuring TNF-α using the "alpha-FNO-IFA-BEST" of "Vektor-Best-Ukraine" (Ukraine) and C-reactive protein (CRP) using the "C-Reactive Protein HS ELISA" kit of "DRG Instruments GmbH" (Germany) using ELISA in accordance with the manufacturers' instructions. Markers of oxidative stress included content of total hydroperoxides (THP), total antioxidant activity (TAA), the level of total superoxide dismutase (T-SOD), which were measured using colorimetric method [5].

Biological age (BA) was calculated to assess the rate of aging in all patients according to the tho different methods that can be used in routine practice. They included the calculation of BA according to the data of objective examination (Voitenko V.P. and others, 1989) (BA1) [6] and according to phenotypic age calculation (Levin M.E. et al., 2018) (BA2) [7]. The aging rates were determined by estimation of the difference between the actual and appropriate BA (or CA, according to the method) (ΔBA). *SIRT1* was measured as an additional marker of aging processes using ELISA.

Molecular genetic research included the determination of the C/G polymorphism (rs7069102) of the *SIRT1* by real-time polymerase chain reaction. CFX Manager Software was used for allelic discrimination.

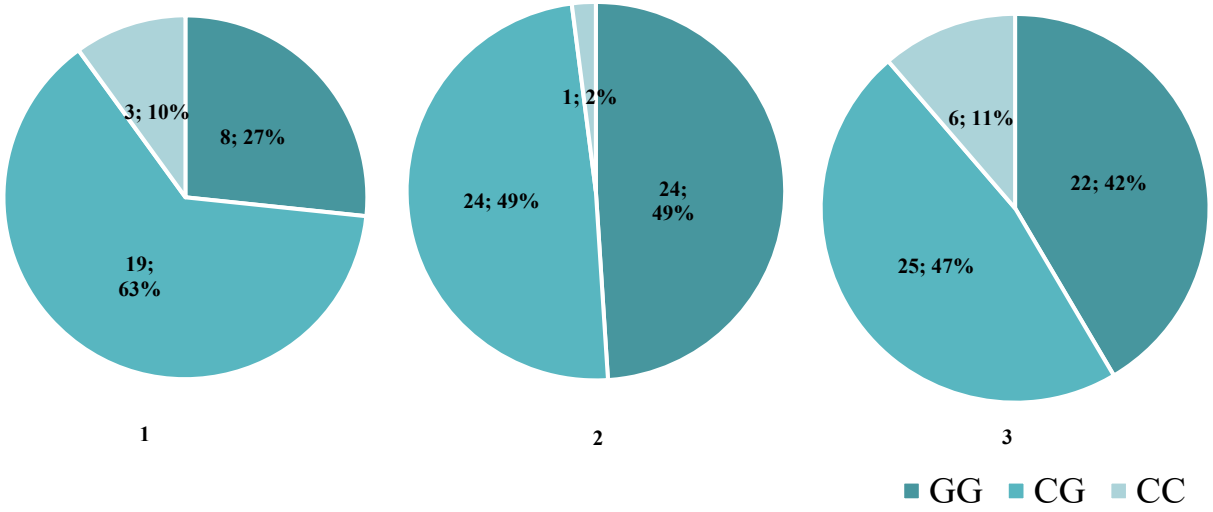
The research comply with the principles of bioethics set out in the Helsinki Declaration "Ethical principles of medical research with people" and "Universal Declaration on Bioethics and Human Rights (UNESCO)", and also approved by the Commission on biomedical ethics of L.T. Mala Therapy National Institute of the National Academy of Medical Sciences of Ukraine. All patients gave informed consent to conduct the necessary research methods.

Statistical processing of the results was performed using the statistical data analysis software package STATISTICA (GRDKR-JFFPD-B34B-3GBV9-QTTHJ No. X12-53766). The Mann-Whitney U test was used to estimate the difference between the two independent samples. The results are presented in the form of the median, lower and upper quartiles – Me (Q1; Q3). The Kruskal-Wallis test was used to determine the influence of different genotypes and alleles on the studied parameters and rates of aging. Evaluation of the reliability of differences in the

frequency of occurrence of traits was performed using Pearson criterion (χ^2). The difference in parameters was considered statistically significant at $p<0.05$ [8].

RESULTS AND DISCUSSION

The carrier frequency of *SIRT1* rs7069102 gene polymorphism was 8% for CC genotype, 51% for CG genotype and 41% for GG genotype in the selected sample of patients. Analysis of the obtained data showed that the frequency of G allele carriers was higher in patients with AH with/without SH compared to the controls (χ^2=14.6; p=0.001). There was a noticeable decrease in frequency of CG carriers and increase in frequency of GG carriers among patients with AH with/without SH compared to the controls (Fig.). There was a significant difference in the prevalence of CC homozygotes and carriers of the G allele between groups of patients with AH depending on the SH presence ($p<0.001$), indicating the probable influence of the studied *SIRT1* polymorphism on the development of thyroid disorders in patients with combined course of AH and SH. It is also impossible to exclude the probable cardioprotective effect of SH due to the higher prevalence of CC genotype among patients with AH and SH compared with AH patients without SH ($p<0,05$).



1 – control group, 2 – patients with AH, 3 – patients with AH and SH. The data are presented as "n; %"

The frequency of genotypes of *SIRT1* rs7069102 gene polymorphism among patients of different groups

We analyzed differences in the values of studied parameters between patients of the same group (with AH with/without SH) with different genotypes in order to identify the polymorphic variant of *SIRT1* most associated with changes in metabolic parameters or markers of inflammation and oxidative stress. There was a significant difference between carriers of CG and GG genotypes in group of patients with AH and without SH in the levels of low-density lipoprotein cholesterol (LDL-C) (3.00 [2.31; 4.09] mmol/L vs 3.98 [3, 65; 4.72] mmol/L, p=0.032) and T-SOD (49.33 [47.21; 51.55] U/ml vs 46.61 [45.24; 49.26] U/ml, p=0.043). The data indicated an increase in cardiovascular risk in patients with AH who had GG genotype due to deterioration of lipid profile, as well as disturbances of redox balance due to reduced antioxidant defense. Such alterations may cause accelerated aging of patients with AH being a carrier of the G allele of *SIRT1* rs7069102 polymorphism. In patients with a combined course of AH and SH differences between carriers of different genotypes were found in the levels of lipid, carbohydrate profile, liver and kidney function and marker of inflammation (Table). Carriers of the CC genotype who had AH and SH had lower insulin resistance and better LDL-C, which is a key component of the risk of cardiovascular mortality compared with carriers of other genotypes, which indicates a protective cardiometabolic effect of CC genotype of *SIRT1* rs7069102 polymorphism. GG homozygotes with AH and SH compared with heterozygotes had worse liver and renal function, as well as greater activity of inflammatory processes, a marker of which was CRP. Given the obtained results, it can be assumed that the G allele is a risk allele in patients with AH, especially with the combined course of AH and SH, which may further contribute to the aging rates of this category of patients.

Comparative characteristics of patients with AH and SH depending on the *SIRT1* rs7069102 polymorphic variant (Me [Q1; Q3])

Parameters	Polymorphic variant of *SIRT1*		p
	CC genotype	CG genotype	
Insulin, μIU/mL	16.24 [12.17;18.00]	29.06 [22.33;36.53]	0.016
HOMA IR	3.56 [2.86;3.95]	7.22 [4.63;8.80]	0.016
	CC genotype	GG genotype	
LDL-C, mmol/L	3.03 [2.49;3.83]	4.34 [3.66;4.88]	0.036
	CG genotype	GG genotype	
Alkaline phosphatase, U/L	1376 [1234;1511]	1710 [1348;1978]	0.022
Uric acid, μmol/L	316 [270;357]	264 [219;289]	0.010
GFR, ml/min/1.73 m²	98 [88;119]	87 [82;95]	0.020
CRP, g/L	2.7 [1.9;4.0]	4.0 [3.1;7.1]	0.025

Similar results were obtained by Habieb M.S. et al. (2021), namely carrying the variant-type G allele of the *SIRT1* rs7069102 was linked to a higher LDL-C, total cholesterol and blood glucose in the Egyptian population [9]. In the Japanese population, carriers of the G allele also had significantly higher levels of total cholesterol and LDL-C compared to the CC genotype carriers [10]. In another study, it was found that GG homozygotes had a significantly higher levels of LDL-C than heterozygotes, which additionally testifies to the negative effect of carrying the G allele on lipid metabolism [11]. Opposite findings on the association of this polymorphism with renal function were obtained in retrospective association study of 724 Caucasian patients who had type 2 diabetes mellitus. It was found that patients with the CC genotype according to both the codominant and recessive models of inheritance are significantly more likely to develop diabetic nephropathy [12].

The association between the *SIRT1* rs7069102 polymorphic variant and indicators of liver function and carbohydrate metabolism was investigated for the first time.

Analysis of BA and aging rates depending on the *SIRT1* polymorphism revealed significant differences only in the group of patients with AH and SH. Patients with the CG genotype showed a significant acceleration of the aging rates according to ΔBA1 compared with patients with the CC genotype (p=0.035). Perhaps CG genotype is the most unfavorable genotype in patients with AH and SH in terms of the aging rates.

Despite the fact that some SIRT1 single-nucleotide polymorphisms showed protective effects for mortality risk, we did not find any research results regarding the association between polymorphic variants of *SIRT1* rs7069102 and rates of aging or biological age in the available scientific literature [13].

Similar results of the role of the *SIRT1* gene polymorphism were obtained by evaluating the influence of different genotypes and alleles on the studied parameters and rates of aging. It was found that SIRT1 genotype affected aspartate aminotransferase (AST) level (H=6.104, p=0.006), and G allele presence affected levels of AST (H=9.813, p=0.004), very low density lipoprotein cholesterol (LDL-C (H=4.871, p=0.036) and high-density lipoprotein cholesterol (HDL-C) (H=4.401, p=0.045) in control group. In patients with AH without SH, SIRT1 polymorphism affected AST (H=3.512, p=0.022), HDL-C (H=3.868, p=0.015), and the G allele presence had an effect on the level of HDL-C (H=7.720, p=0.008). In patients with combined course of AH and SH, SIRT1 polymorphism affected the level of HDL-C (H=2.904, p=0.040), LDL (H=2.788, p=0.047), BA1 (H=2.802, p=0.047); whereas the C allele presence significantly affected the level of HDL-C (H=5.878, p=0.021) and LDL-C (H=6.362, p=0.017), and G allele presence had an effect on BA1 (H=9.572 , p=0.004). Our data indicate a significant role of *SIRT1* polymorphism in the formation of cardiometabolic changes and subsequent disorders of the aging rates in patients with AH with/without SH. Therefore, assessment of the *SIRT1* genotype may be effective in preventing cardiovascular complications and, as a consequence, slowing the aging rates in patients with AH, especially with concomitant SH.

CONCLUSIONS

1. The CG (47%) and GG (42%) genotypes of the polymorphic gene rs7069102 S*irtuin 1* are dominant in patients with arterial hypertension combined with subclinical hypothyroidism.
2. Carriers of the CG genotype of the S*irtuin 1* gene polymorphic marker (rs7069102) with arterial hypertension and subclinical hypothyroidism have significantly higher insulin resistance (p=0.016) compared to carriers of the CC genotype, while carriers of the GG genotype have higher levels of low-density lipoprotein cholesterol (p=0.036) compared with carriers of CC genotype and higher levels of alkaline phosphatase (p=0.022), C-reactive protein (p=0.025) and lower glomerular filtration rate (p=0.020) compared with carriers of CG genotype, that affects the rate of aging progression in this category of patients.
3. Timely detection of a polymorphic variant of the S*irtuin 1* gene may be effective in premature aging prevention in patients with arterial hypertension and subclinical hypothyroidism.

Contributors:

Radchenko A.O. – investigation, resources, formal analysis, writing (original draft), visualization;

Kolesnikova O.V. – conceptualization, supervision, project administration, methodology, writing (editing).

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