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SIRT1 (RS7069102) AND *SOD2* (RS4880) GENETIC VARIANTS AS A MODIFIER OF CARDIOMETABOLIC PROFILE IN PATIENTS WITH ARTERIAL HYPERTENSION AND SUBCLINICAL HYPOTHYROIDISM

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ABSTRACT

Arterial hypertension (AH) and subclinical hypothyroidism (SH) are a common example of comorbid diseases. They share common cardiovascular risk factors, among which genetic factors are the least studied, albeit no less important. The aim of our study was to evaluate the role of *SOD2* (rs4880) and *SIRT1* (rs7069102) polymorphisms as determinants of the development of cardiometabolic disorders among patients with AH depending on the presence of SH. The study included 79 patients with stage I-II grade 1-2 AH with a median age of 49.4 [41.4;55.1] (women 63%), who were divided into 2 groups depending on the presence of SH . The study presents the distribution of genotypes and alleles of the investigated polymorphisms, as well as the levels of cardiometabolic indicators in patients of different groups depending on the polymorphic gene variant. According to our study carriers of the G allele of the SIRT1 gene and the C allele of the SOD2 gene with a combined course of AH and SH have higher levels of insulin (p=0.021), homeostatic model assessment for insulin resistance (p=0.012), glycosylated hemoglobin (p=0.000), total cholesterol (p= 0.049), non-high-density lipoprotein cholesterol (p=0.026), very low-density lipoprotein cholesterol (p=0.036), C-reactive protein (p-0.000) and tumor necrosis factor alpha (p=0.000), as well as a higher frequency of elevated alanine aminotransferase levels (p=0.012), insulin (p=0.027) and C-reactive protein (p=0.017) compared to euthyroid patients with similar genotypes.

1. Introduction

The steady increase in the life expectancy of people in the world contributes to the increase in the frequency of comorbid diseases, the most common of which is a combination of cardiovascular and endocrine pathology. Arterial hypertension (AH) is one of the most common cardiovascular disease (CVD) and it is often associated with a large number of complications, high rates of disability and mortality, that determines the high need for continuous improvement of treatment and preventive measures among patients with AH [1].

On the other hand, subclinical hypothyroidism (SH), which is often detected among hypertensive patients as a result of screening, is often not only a concomitant pathology, but also one of the risk factors for the occurrence of AH due to its adverse effect on blood pressure.

In addition, there are many data available indicating the development of a worse cardiometabolic profile in case of the combined course of AH and SH compared with isolated AH.

The probable reasons for such changes are an increased negative impact on the development of dyslipidemia, mainly hypercholesterolemia, the effect on thyroid-stimulating hormone (TSH) receptors in liver cells, an increase in the level of pro-inflammatory cytokines, the development of oxidative stress due to the direct effect of TSH mainly on the NF-κB signaling pathway and development of insulin resistance as a result of

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inhibition of the expression of insulin receptor 1 substrate protein and tyrosyl phosphorylation in adipocytes under the influence of TSH [2, 3].

However, the presence of cardiovascular risk (CVR) factors, such as smoking, overweight, low physical activity, and others, can significantly increase the severity and progression of cardiometabolic disorders. CVR factors, in addition to traditional ones, also include redox balance disorders and genetic risk factors [4]. Several genetic loci have already been identified that contribute to the occurrence of AH. However, new genetic risk factors, especially genome-wide associations, need to be identified. A number of scientific research results indicate that *SIRT1* rs7069102 and *SOD2* rs4880 polymorphisms can be such risk factors for the development of CVD due to their influence on metabolism and redox processes in the body.

Thus, Kilic U. et al. (2014) found that G allele of rs7069102 C > G in a Turkish population was associated with increased by 2.4 times risk for stable coronary artery disease [5]. Later, the authors showed that homozygous mutant GG genotype and a mutant G allele for rs7069102 were more common among patients with atherosclerosis compared to control group ($p<0.001$) [6]. Moreover, this study showed that carriers of the G allele had significantly higher levels of total antioxidant status (TAS) compared to controls, which, unlike the group of patients with the CC genotype, were not improved by statin therapy. This may indicate an additional mediated negative effect of G allele on the metabolic profile due to a disturbance in the redox balance. Yamac A. H. et al. (2019) proved that carriers of the G allele with myocardial infarction in the same population had a worse cardiometabolic profile, namely enhanced body mass index, elevated levels of triglycerides, very low-density lipoprotein (VLDL) compared to wild-type CC genotype and worse fasting blood glucose compared to controls [7]. According to the results of the study among Japanese individuals (Shimoyama Y. et al., 2011), male GG carriers of rs7069102 had increased body fat ratio and systolic blood pressure [8]. Among Slovene (Caucasian) patients with type 2 diabetes, the rs7069102 polymorphism was associated with the development of diabetic nephropathy, although the CC genotype was a risk factor [9]. Unfortunately, most of the presented results are not up-to-date, and we did not find more recent research on this issue. In addition, the results conducted in different populations are quite contradictory.

SOD2 rs4880 gene is the most studied single nucleotide polymorphism among all polymorphic variants of this gene. Therefore, the knowledge about the role of this polymorphism in the formation of CVR is wider, but still some of the results remain contradictory and differ depending on the source. For example, the C allele, especially the CC genotype, was associated with a higher risk of developing chronic kidney disease and diabetic nephropathy in patients with type 2 diabetes among Iran residents and Chinese Han population [10, 11]. Opposite results were obtained among the Malaysian population of three different ethnic groups (Chinese, Malays and Indians), namely the authors established that C allele of *SOD2* rs4880 had significant protective effects on the risk of diabetic nephropathy [12]. It was also observed that C allele was more common among Iranian women with preeclampsia [13], which may indicate a possible connection of this allele with the risk of developing AH in the future. However, a recent meta-analysis found no association between *SOD2* rs4880 polymorphism and preeclampsia in Asians, Caucasians or Middle Easterners [14].

Decharatchakul N. et al. (2019) found that *SOD2* rs4880 CC genotype contributes to increased risk of dyslipidemia related to atherosclerotic severity [15]. In addition, Lewandowski Ł. et al. (2020) suggested that this polymorphism might be a hereditary factor for developing obesity due to high prevalence of *SOD2* rs4880 TC genotype (90%) among obese group of patients [16]. However, it was in the non-obese group that the authors noted a significant increase in levels of fasting glucose, insulin and insulin resistance among patients with the TT genotype compared to patients with the C allele. Therefore carrying different alleles of *SOD2* gene can also have different effects on the development of CVD depending on the studied population and concomitant diseases but available date is still limited.

Based on scientific data on the role of *SIRT1* rs7069102 and *SOD2* rs4880 polymorphisms in the development of oxidative stress and the formation of CVR, we hypothesized that the combination of mutant alleles of these polymorphisms in patients with the combined course of AH and SH can significantly increase the severity and frequency of cardiometabolic disorders compared to patients with isolated course of AH. In addition, it seems necessary to determine the prevalence of selected genotypes in the Ukrainian population and the allele or genotype of CVR in it.

That is why, the aim of our study was to evaluate the role of *SOD2* (rs4880) and *SIRT1* (rs7069102) polymorphisms as determinants of the development of cardiometabolic disorders among patients with AH depending on the presence of SH.

2. Material and methods

Study Design and Study subjects

The study included 79 patients with a median age of 49,4 [41,4;55,1] (women 63%), 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 – 43 euthyroid patients; group 2 – 36 patients with SH. Written informed consent was received from all individuals who were included in the study. This study was approved by the Commission on biomedical ethics of L.T. Mala Therapy National Institute of the National Academy of Medical Sciences of Ukraine (permission number 11, 17 October 2019). AH was diagnosed based on the 2018 ESC/ESH Guidelines for the management of AH. The diagnosis of SH was made according to the ETA Guidelines (2013).

Immunoenzyme techniques and biochemical research methods

Blood collection for enzyme-linked immunosorbent assay (ELISA) and biochemical assay was carried out from the ulnar vein with minimal tourniquet tightening into vacuum tubes (vacutainers) with gel and coagulation activator to obtain serum, and with K3EDTA – for blood plasma. Blood serum for ELISA was stored at minus 20 ºC for no longer than 6 months, and for biochemical studies – at minus 70 ºC for no longer than 6 months.

To assess the cardiometabolic profile, we determined routine biochemical blood parameters, as well as indicators of a proinflammatory state.

Lipid (TC, total cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol), carbohydrate profile (FPG, fasting plasma glucose, insulin), kidney (uric acid, creatinine, albumin) and liver (AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase) function were performed according to the generally accepted methods. The content of insulin, pro-inflammatory markers, namely C-reactive protein (CRP) and tumor necrosis factor α (TNFα), markers of thyroid function, namely thyrotropin-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), was assessed by ELISA using appropriate kits in accordance with the manufacturers' instructions: «Insulin ELISA» and «C-Reactive Protein HS ELISA» of «Instruments GmbH» (Germany), «HS-TNF-α Accquant ELISA Kit» (Wuhan Fine Biotech Co., China), «ТТГ-ІФА», « Вільний Т3-ІФА», «Вільний Т4-ІФА» of «ХЕМА» (Ukraine). Optical density was measured on a microplate ELISA reader HTI ImmunoChem-2100 (USA). Additionally, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and glomerular filtration rate (GFR) were calculated in all patients based on obtained parameters.

Molecular genetics techniques

A total DNA was extracted from whole blood from peripheral blood samples from each participant using kits "NeoPrep50" ("Неоген", Ukraine) and stored before amplification at minus 20 oC for no longer than 6 months.

Genotyping of polymorphisms rs7069102 of the sirtuin gene (SIRT1) and rs4880 of the superoxide dismutase gene (SOD2) was performed using TaqMan technology (allele-specific polymerase chain reaction with real-time detection of the result). The study used TaqMan SNP Genotyping Assay for *SIRT1* and *SOD2* polymorphisms (assays C_1340389_10 and C_8709053_10 accordingly) and Universal PCR Master Mix (Ref. 4304437) (Thermo Fisher Scientific, USA) in accordance with the TaqMan® Universal PCR Master Mix USER GUIDE (Applied Biosystems by Life Technologies, USA). Amplification was performed using the CFX96 Touch Real-Time PCR Product Detection System (BioRad, USA). CFX Manager Software (USA) was used for allelic discrimination.

Genotyping of polymorphisms namely rs7069102 of the sirtuin gene (SIRT1) and rs4880 of the superoxide dismutase gene (*SOD2*) was performed using TaqMan technology (allele-specific polymerase chain reaction with real-time detection of the result). We used TaqMan SNP Genotyping Assay for *SIRT1* and *SOD2* polymorphisms (assays C_1340389_10 and C_8709053_10 accordingly) and Universal PCR Master Mix (Ref. 4304437) (Thermo Fisher Scientific, USA) in accordance with the TaqMan® Universal PCR Master Mix USER GUIDE (Applied Biosystems by Life Technologies, USA). Amplification was performed using the CFX96 Touch Real-Time PCR Product Detection System (BioRad, USA). CFX Manager Software (USA) was used for allelic discrimination.

Characteristics of studied gene polymorphisms presented in table 1. C allele was considered to be the wild-type allele of the *SIRT1* polymorphism (rs7069102, C>G).

Its prevalence is known to be 0.32717 in the European population. G allele was mutant allele for all analyzes in our study.

The mutation with this polymorphism occurs in an intron, so the polymorphism does not affect the synthesis of amino acids. The reference allele of the *SOD2* polymorphism (rs4880, T>C) was T allele, the prevalence of which in the European population is 0.501909. C allele was mutant allele for all analyzes in our study. rs4880 is a missense variant in exon 2: in the presence of the T allele, the amino acid valine (Val) is encoded in codon 16, while in the case of the polymorphic variant (the presence of the C allele), the amino acid alanine (Ala) is encoded instead (based on data procured from Single Nucleotide Polymorphism database (dbSNP) at the National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Software package STATISTICA (GRDKR-JFFPD-B34B-3GBV9-QTTHJ serial № X12-53766) was used to calculate the significance of the studied variables. Normal distribution of data was tested with Kolmogorov–Smirnov test. The total number (n) and the percentages for genotypic distribution have been calculated. The demographic characteristics are described as median, lower and upper quartiles – Me [Q1; Q3] for continuous variables, and as n and/or percentages for categorical variables. Genotyping results were examined for any deviation from the Hardy–Weinberg Equilibrium (HWE) via Chi-squared test calculation. This test was also used in order to test differences between the groups for categorical variables. The Mann–Whitney U test was used to compare continuous variables between the two independent samples. The p-value < 0.05 was set as the statistically significant threshold.

Single nucleotide polymorphism	Nucleotide substitution	Chromosomal position	Amino acid substitution	Forward 5'-3' and Reverse 3'-5' PCR primers
SIRT1/SIRT1 gene				
rs7069102	C>G	Chr.10:67903362	N/A (Intron Variant)	F: AGAAGAAAGAAAGGCA TAATCTCTG R: AGAAAAGCCATTATTTC TGCAGATA
SOD2/SOD2 gene				
rs4880	T>C	Chr.6:159692840	Val > Ala (missense variant in exon 2)	F: CTGCCTGGAGCCCAGAT ACCCCAAA R: CCGGAGCCAGCTGCCTG CTGGTGCT

Table 1. Characteristics of studied gene polymorphisms.

3. Results

The basic demographic and clinical characteristics of the AH patients with/without SH are presented in Table 2. Although the differences are not significant, patients with a combined course of AH and SH are older, overweight and mostly presented by women compared to euthyroid patients with AH.

Characteristic	AH (n=43)	AH+SH (n=36)	p-value
Age, years	47.9 [41.3;54.9]	50.6 [42.0;56.4]	0.485
Gender (female), n (%)	24 (55.8)	26 (72.2)	0.132
BMI, kg/m²	27.5 [25.1;29.7]	28.1 [26.8;30.3]	0.181
Systolic BP, mm Hg	130 [120;140]	125 [120;138]	0.418
Diastolic BP, mm Hg	82 [75;90]	81 [71; 90]	0.412
Pulse pressure, mm Hg	48 [40;54]	45 [40;52]	0.671

Table 2. Demographic and clinical characteristics of euthyroid patients with AH and patients with AH and SH. Notes: AH, arterial hypertension; AH+SH, arterial hypertension with subclinical hypothyroidism; BMI, body mass index; BP, blood pressure

The study results of the frequencies of *SOD2* T>C (rs4880) and *SIRT1* C>G (rs7069102) polymorphism genotypes in different groups are presented in Table 3. The distribution of the genotypes of the studied polymorphisms corresponded to the Hardy–Weinberg equilibrium (HWE) ($p > 0.05$) both for cases in the group with the combined course of AH and SH, and in the group with the isolated course of AH. No statistically significant differences were found between the frequencies of polymorphism genotypes of the studied groups. There were only a few patients with the CC genotype in the SIRT1 gene in both groups.

Variables	AH, n (%)	AH+SH, n (%)	p-value
SIRT1 (rs7069102)			
Genotypes			
C/C	1 (2.3)	4 (11.1)	-
C/G	21 (48.8)	17 (47.2)	0.138
G/G	21 (48.8)	15 (41.7)	0.107
Alleles			
C	23 (26.7)	25 (34.7)	0.524
G	63 (73.3)	47 (65.3)	0.110
SOD2 (rs4880)			
Genotypes			
T/T	9 (20.9)	11 (30.6)	-
T/C	27 (62.8)	18 (50)	0.272
C/C	7 (16.3)	7 (19.4)	0.774
Alleles			
T	45 (52.3)	40 (55.6)	0.714
C	41 (47.7)	32 (44.4)	0.327

Table 3. Distribution of genotypes and alleles of T>C (rs4880) of the SOD2 gene and C>G (rs7069102) of the SIRT1 gene polymorphisms in the groups of euthyroid patients with AH and patients with a combined course of AH and SH (*as a percentage of the number of people in this group)*

Data on the genotypes combination of rs7069102 C>G and rs4880 T>C polymorphisms in patients with AH depending on the presence of SH are presented in Table 4. No significant difference was found in the frequency of genotypes of these polymorphisms in the studied patients. There were no patients with AH being homozygous for wild-type allele in both polymorphisms. Also, an extremely low occurrence of such genotypes in the group of patients with a combined course of AH and SH is noted.

Among our patients with the most frequent variant of the *SIRT1* gene, namely CG and GG, we studied changes in the cardiometabolic profile. We compared homozygotes (TT) with carriers of the C-allele of the *SOD2* gene in groups of patients with AH depending on the SH presence.

We also compared patients of different groups with the same genotypes in order to determine whether the presence of concomitant SH on the background of AH determines the greater severity of cardiometabolic changes depending on the *SOD2* genetic variant (Table 4).

All subgroups were age matched ($p>0.05$). There were no abnormalities in the levels of markers of the liver and kidney functional state. Carriers of the G-allele of *SIRT1* with a combined course of AH and SH, had significantly higher levels of HbA1c and TNFα compared to euthyroid patients with AH and a similar *SIRT1* genotype regardless of the *SOD2* genotype. Carriers of mutant alleles in both genes with AH and concomitant SH had a greater number of indicators that were worse compared to euthyroid patients with AH. Thus, in addition to existing deteriorations in HbA1c and TNFα levels, patients with AH and SH had worse levels of insulin, HOMA IR, TC, non-HDL-C, VLDL-C, and CRP.

Polymorphisms: C>G / T>C	AH, n (%)	AH+SH, n (%)	χ2	p-value
C/C / T/T	0 (0)	2 (5.6)	3.526	0.317
C/C / T/C + C/C	1 (2.3)	2 (5.6)		
C/G + G/G/ T/T	9 (20.9)	9 (25.0)		
C/G + G/G/ T/C +C/C	33 (76.7)	23 (63.9)		

Table 4. Distribution of genotypes when combining T>C (rs4880) of the SOD2 gene and C>G (rs7069102) of the SIRT1 gene polymorphisms in patients with AH depending on the SH presence

Parameters	AH		AH+SH		p-value
	T/T rs4880 (n=9)	T/C + C/C rs4880 (n=33)	T/T rs4880 (n=9)	T/C + C/C rs4880 (n=23)	
Carbohydrate profile					
Insulin, μIU/mL	21.04 [15.28;31.97]	18.32 [12.75;26.21]	23.05 [18.57;27.05]	26.41 [20.84;35.83]	**0.021²**
HOMA IR	4.02 [3.62;7.89]	4.50 [3.02;6.72]	4.60 [4.42;6.67]	8.26 [4.26;9.39]	**0.012²**
HbA1c, %	5.27 [5.15;5.72]	5.28 [5.09;5.63]	6.16 [5.48;6.23]	5.87 [5.76;6.17]	**0.024¹ 0.001²**
Lipid profile					
TC, mmol/L	6.12 [5.36;6.63]	5.59 [4.72;6.31]	5.82 [4.96;6.41]	6.10 [5.38;7.10]	**0.049²**
Non-HDL-C, mmol/L	4.92 [3.98;5.32]	4.27 [3.46;4.93]	4.46 [3.93;5.18]	5.01 [4.05;5.91]	**0.026²**
TG, mmol/L	1.28 [0.95;1.55]	1.38 [0.89;1.95]	1.48 [1.07;1.80]	1.67 [1.20;2.35]	0.052²
VLDL-C, mmol/L	0.60 [0.41;0.85]	0.62 [0.40;0.88]	0.67 [0.49;0.87]	0.72 [0.57;1.06]	**0.036²**
Proinflammatory state					
CRP, g/L	1.9 [1.5;4.6]	1.5 [1.3;3.6]	3.70 [2.4;5.2]	3.2 [2.3;7.1]	**0.001²**
TNFα, pg/mL	1.9 [1.6;2.0]	2.2 [1.8;2.7]	3.8 [3.5;4.7]	4.24 [3.62;4.92]	**0.001¹ 0.001²**

Table 5. Characteristics of the cardiometabolic profile of patients carrying the G-allele of the C>G polymorphism (rs7069102) of the SIRT1 gene in combination with the T/T and T/C+C/C genotypes of the T>C polymorphism of the SOD2 gene. Notes: FPG, fasting plasma glucose; TC, total cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; GFR, glomerular filtration rate; 1 – T/T among patients with AH vs T/T among patients with AH+SH; 2 – T/C + C/C among patients with AH vs T/C + C/C among patients with AH+SH

Among carriers of the G-allele in the SIRT1 gene within the same group (AG or AG+CG) the presence of a mutant *SOD2* allele was not accompanied by a significant increase in the proportion of patients with cardiometabolic parameters outside the normal range compared to carriers of the TT genotype.

Among the carriers of the G-allele in the SIRT1 gene and rs4880-TT genotype, no significant differences were observed among patients of different groups (AG and AG+SG). However, carriers of both mutant variants of genes and the combined course of AH and SH significantly more often had elevated levels of ALT (60.9% vs 27.3%, $\chi2$=6.321, p=0.012), insulin (56.5% vs 27.3% , $\chi2$=4.861, p=0.027) and CRP (34.8% vs 9.1%, $\chi2$=5.668, p=0.017) compared to euthyroid patients with AH.

4. Discussion

In our study we found that the presence of SH among AH patients was accompanied by slight increase in the number of women, median age and body mass index, though such differences in basic characteristics between two groups of patients were not significant. This can be likely due to the fact that older age and female gender are risk factors for the development of SH, while weight gain may be an indicator of the future development of such a common manifestation of overt hypothyroidism as obesity.

We did not find any differences in the frequency of genotypes among patients with AH depending on the presence of concomitant SH. However, there were almost no patients with the CC genotype of the *SIRT1* gene among our patients. This coincides with the fact that such a genotype is also not common in the general population and in available study populations in other researches [5-9, 17]. The frequencies of the T and C alleles of the *SOD2* gene in our study are similar and coincide with the results of the prevalence of the wild-type T allele in the general European population. It should be noted that depending on the selected population, the risk allele for the development of oxidative disorders may change. For example, in the Asian population, the risk allele is the T allele, the C allele is usually considered as an allele associated with an adverse effect on the processes of oxidative stress in the European population, and heterozygous genotype, which was the most common, was associated with a positive effect on the redox balance in a separate study among the Southeastern Caucasian European population [12, 16, 18].

Since in our study the number of patients with the CC genotype of the *SIRT1* gene was insufficient to determine the characteristics of cardiometabolic indicators depending on the combination of polymorphic variants of the *SIRT1* and *SOD2* genotypes, we evaluated only patients who were carriers of the G allele of *SIRT1* gene. Among the selected patients, we compared carriers of the C allele as a probable risk allele in the European population with carriers of the TT genotype. We found that the levels of HbA1c, TNFα, HOMA IR, TC, non-HDL-C, VLDL-C and CRP were worse among patients with AH in the presence of SH, but this was true only among carriers of both mutant alleles of *SIRT1* and *SOD2* genes. Although within the same group (AH or AH + SH) carriers of G allele in SIRT1 gene had no significant differences in the indicators of the cardiometabolic profile based on *SOD2* genotypes, the non-significant worsening of indicators of carbohydrate, lipid profile and pro-inflammatory state was observed only in patients with a combined course of AH and SH. Such results indicate that the presence of a mutant allele of *SOD2* in *SIRT1* mutant allele carriers is accompanied by a worsening of the cardiometabolic profile, mainly markers of carbohydrate and lipid metabolism, as well as a pro-inflammatory state, especially in case of concomitant SH on the background of existing AH.

In contrast to the results of other authors [9-12], we also did not find any differences in the functional indicators of the kidneys among the studied patients depending on the polymorphic gene variants. We assume that such results of other researchers namely significant changes in kidney function associated with different genotypes, may be directly related to the impact of existing type 2 diabetes. In addition, it is likely that the severity of such changes may become noticeable in patients with a long course of AH and SH, that was not taken into account in the current study.

The limitations of the conducted study may be a small sample of studied patients, as well as the fact that we assessed only one of the known polymorphic variants of the *SIRT1* and *SOD2* genes in our study. Therefore, it can be assumed that the differences in the general effect of selected polymorphic variants of these genes on CVR are related to the different impact on the activity and expression of SIRT1 and SOD2 accordingly, that depends, on the one hand, on the studied population, and on the other hand, on the diverse contribution of selected polymorphisms on the synthesis and function of SIRT1 and SOD2 compared to the impact of other polymorphic variants of the same genes.

5. Conclusions

The most frequent genotypes and alleles among patients with arterial hypertension are the G-allele of the *SIRT1* gene and the TC genotype of the *SOD2* gene, however, none of the studied polymorphisms is associated with the risk of developing subclinical hypothyroidism.

G-allele of the *SIRT1* gene and C-allele of the *SOD2* gene being present at the same time has an adverse effect on the cardiometabolic profile of patients with a combined course of arterial hypertension and subclinical hypothyroidism, which is not observed in patients with an isolated course of arterial hypertension. Carriers of the G-allele of the *SIRT1* gene and the C-allele of the *SOD2* gene with a combined course of arterial hypertension and subclinical hypothyroidism have higher levels of insulin (p=0.021), homeostatic model assessment for insulin resistance (p=0.012), glycosylated hemoglobin (p=0.000), total cholesterol (p= 0.049), non-high-density lipoprotein cholesterol (p=0.026), very low-density lipoprotein cholesterol (p=0.036), C-reactive protein (p=0.000) and tumor necrosis factor alpha (p=0.000), as well as a higher frequency of elevated alanine aminotransferase levels (p=0.012), insulin (p=0.027) and C-reactive protein (p=0.017) compared to euthyroid patients with similar genotypes.

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