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The human hypoxia-inducible factor 1alpha gene in anthracycline-induced heart failure

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SOUHRN

Cíl: Cílem studie bylo zhodnotit úlohu genu pro hypoxií indukovatelný faktor 1alfa (hypoxia-inducible factor 1alpha, HIF1A; gen 1772C>T, rs11549465) v antracyklinem vyvolaném srdečním selhání (anthracycline-induced heart failure, AIHF) u žen bez kardiovaskulárního onemocnění (KVO) v předchozí 24 měsících.
Metody: Do studie bylo zařazeno celkem 114 žen s mediánem věku 47,0 (44,0; 52,0) roku s AIHF stupně I–III NYHA, jimž byl podáván doxorubicin pro karcinom prsu. Při vstupu do studie u nich bylo s použitím polymerázové řetězové reakce provedeno vyšetření na genový polymorfismus.
Výsledky: Po 24měsíčním sledování vykázaly všechny pacientky remisi karcinomu prsu; byly rozděleny do dvou skupin: skupinu 1 tvořily ženy s nepříznivým průběhem AIHF (n = 36), skupinu 2 tvořily ženy bez AIHF (n = 75). Přítomnost C/T genotypu s genem pro HIF1A (1772C>T, rs11549465) (OR = 3,65; p = 0,009) souvisela s nepříznivým průběhem AIHF. U žen s C/T genotypem s genem pro HIF1A (1772C>T, rs11549465) byla zjištěna další progrese AIHF: ejekční frakce levé komory se statisticky významně (p < 0,001) snížila o 11,8 % z 51 (47; 53) na 45 (43; 46) %, její end-systolický průměr se zvětšil o 7,8 % (p < 0,001) a end-diastolický průměr o 5,2 % (p < 0,001).
Závěr: Polymorfismus C/T hypoxií indukovatelného faktoru pro gen 1alfa (1772C>T, rs11549465) u žen bez předchozího KVO byl spojen s nepříznivým průběhem AIHF za období 24 měsíců.

Keywords:
Anthracycline-induced heart failure
Genes
Hypoxia-inducible factor 1alpha
Polymorphism
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ABSTRACT

Objective: The objective of the study was to evaluate the role of hypoxia-inducible factor 1alpha (HIF1A) gene (1772C>T, rs11549465) in the course of anthracycline-induced heart failure (AIHF) in women without previous cardiovascular diseases (CVD) during 24 months.
Methods: A total of 114 women, median age of 47.0 (44.0; 52.0) years with AIHF of NYHA class I–III who received doxorubicin for breast cancer were enrolled. Evaluation of gene polymorphisms was carried out by polymerase chain reaction at baseline.
Results: After 24 months of follow-up all patients had breast cancer remission and were divided into 2 groups: group 1 comprised women with adverse course of AIHF (n = 36), group 2 comprised those without it (n = 75). The presence of C/T genotype of HIF1A gene (1772C>T, rs11549465) (OR = 3.65; p = 0.009) was related with adverse course of AIHF. Women with C/T genotype of HIF1A gene (1772C>T, rs11549465) had further progression of AIHF: left ventricle ejection fraction significantly (p <0.001) decreased by 11.8% from 51 (47; 53) to 45 (43; 46)%, end-systolic dimension increased by 7.8% (p <0.001), and end-diastolic dimension by 5.2% (p <0.001).
Conclusion: Polymorphism of C/T of hypoxia-inducible factor 1alpha gene (1772C>T, rs11549465) in women without previous CVD was associated with adverse course of AIHF during 24 months.

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Introduction

Anthracyclines are important components of many chemotherapy regimens; but their using is associated with the left ventricular dysfunction and heart failure (HF) that can occur up to decades after exposure.[1] Nevertheless, the mechanisms underlying cardiotoxic drug effects are still poorly understood and controversial. It is well-established that doxorubicin (DOX) exerts their anticancer action by directly targeting and inhibiting topoisomerase-2 in cancer cells.[2] However, the same mechanism can hardly explain the toxic effect of DOX on the heart and vessels; therefore, recently there are data on the involvement of endothelial dysfunction, apoptosis, extracellular matrix remodeling, and oxidative stress in the pathogenesis of cardiotoxic damage.[3]

In last years it has been established that anthracyclines inhibit binding of the hypoxia-inducible factor 1alpha (HIF1A)/aryl hydrocarbon receptor nuclear translocator (ARNT) heterodimer to the target gene enhancer and reduce the cellular hypoxia response.[4] HIF1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating the gene transcription involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia.[5] It is composed of an oxygen-labile alpha-subunit and a constitutively expressed beta-subunit.[4] HIF1A is a universal regulator for hypoxia-inducible gene expression, and it can be activated by oxidative stress, inflammation, and fibrosis, actions that are opposed by HIF2A.[6] Imbalances in HIF1A and HIF2A may contribute to the progression of chronic HF, atherosclerotic and hypertensive vascular disorders, and chronic kidney disease.[7] These disorders are characterized by activation of HIF1A and suppression of HIF2A, which may be potentially related to mitochondrial and peroxisomal dysfunction.[8]

It has been demonstrated that DOX treatment induces significant changes in the expression of genes controlling both mitochondrial structure and metabolism,[9] as well as DOX, preferentially accumulate in the mitochondria of cardiomyocytes, strongly impacting on both the structure and the activity of these organelles that cause the increased ROS production.[10] Anthracycline-mediated production of these reactive species in turn may potentially lead to imbalances in HIF1A and HIF2A, and as a consequence progression of heart dysfunction induced by anthracyclines. However, if the hypoxia caused during treatment with anthracyclines is quite understandable, then it is not clear whether it affects the development and progression of HF in the long-term periods after chemotherapy treatment.

Thus, the objective of the study was to evaluate the role of hypoxia-inducible factor 1alpha gene (1772C>T, rs11549465) in the course of anthracycline-induced HF (AIHF) in women without previous cardiovascular diseases (CVD) during 24 months.

Methods

Ethical disclosure

The study was conducted in accordance with the Declaration of Helsinki for all human research and was approved by the local Ethics Committee of Cardiology Research Institute, Tomsk National Research Medical Center (protocol №207, 23 Dec 2020). Informed written consent was obtained from all patients before their enrollment in this study.

Study population

This was a prospective, observational, single-center trial. At the 12 months after chemotherapy completion a total of 114 women (median age of 48.0 [46.0; 52.0] years) with AIHF were enrolled. The control group comprised the women (n = 70) who received doxorubicin as part of chemotherapy for breast cancer, but did not develop AIHF (median age of 45.0 [42.0; 50.0] years). The absence of cardiac pathology was confirmed by anamnesis, electrocardiography, echocardiography, and coronary angiography.

Inclusion criteria: 1) breast cancer women without prior cardiovascular diseases who develop AIHF of New York Heart Association (NYHA) class I–III; 2) cancer treatment received: either a combination of doxorubicin and cyclophosphamide (AC regimen), or combination of doxorubicin, cyclophosphamide and docetaxel (TAC regimen); 3) NT-proBNP levels ≥125 pg/mL; 4) breast cancer remission.

Exclusion criteria: 1) diabetes mellitus types 1 and 2; 2) coronary heart disease; 3) hypertension; 4) valve defects and prior cardiomyopathies of any etiology; 5) heart failure with an alternative cause of manifestation (severe lung disease, a history of primary pulmonary hypertension, anemia, body mass index> 50 kg/m^2); 6) concomitant severe renal, hepatic, or multiple organ failure; 7) indications of poor drug tolerance; 8) chronic alcoholism or mental disorders; 9) ovarian pathology or hormonal imbalance.

Criteria for the development of AIHF were left ventricle ejection fraction (LVEF) absolute decrease higher than 10% points from values before chemotherapy initiation or less than 55% with signs/symptoms of HF and NT-proBNP levels ≥125 pg/mL at the 12 months after chemotherapy completion.

Blood sampling and biochemical analyses

Blood samples were obtained by venipuncture and adequate centrifugation serum samples were stored at –24 °C with a single freeze–thaw cycle. The serum levels of NT-proBNP were determined using a sandwich immunoassay (NT-proBNP, Biomedica immunoassays, Austria).

Gene polymorphisms

The buccal epithelium was taken to determine gene polymorphism. Evaluation of gene polymorphisms of gene polymorphisms of hypoxia-inducible factor 1alpha (1772C>T, rs11549465) was carried out by polymerase chain reaction at baseline (Fig. 1).

Hardy-Weinberg equilibrium test was used to control the results of genotyping and it was carried out using the online program on the website of the Institute of Human Genetics (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Gene	High Resolution Melt Polymerase chain reaction	Sanger sequencing
HIF1A (1772C>T)	Straight primer: 5′- TCCAGTTACGTTCCTTCGATCA -3′	5′-GGACACAGATTTAGACTTGGAGA-3′
	Reverse primer: 5′-TGAGGACTTGCGCTTTCAGG-3′	5′-ATTCATCAGTGGTGGCAGTG-3′

Fig. 1 – Primer and probe structures used for genotyping by real-time allele-specific amplification using SYBR Green I for fluorescence induction.

Treatment

All patients received betablocker (carvedilol) and angiotensin-converting enzyme inhibitor (enalapril) therapies for the treatment of AIHF, and some of them received loop diuretics for volume management. Doses of drugs were titrated to the maximum tolerated.

Study outcomes and definitions

Adverse course of AIHF was defined as new or worsening symptoms/signs of HF and/or hospitalization due to decompensation of HF requiring intravenous diuretics; LVEF absolute decrease higher than 5% points; an increase in NYHA by 1 or more classes; or development of atrial fibrillation. The atrial fibrillation was diagnosed by electrocardiography as an episode lasting at least 30 seconds.[11] Patients who did meet these criteria had favorable course of AIHF.

Statistical analysis

All analyses were performed with STATISTICA V10.0 software package or R software, version 2. The sample data were not normally distributed; therefore, nonparametric analyzes were used. Categorical variables are expressed as counts and percentages. Continuous variables are expressed as the median (25th–75th percentiles [P25-75]). Statistical differences between two groups were compared using the $\chi 2$ test for categorical variables and using Mann–Whitney U test for continuous variables or Kruskal–Wallis test for ≥3 groups. Odds ratios (OR) for gene polymorphisms were determined from logistic regression models. OR = 1 implies no association between genotype and a course of AIHF, OR >1 implies that genotype is associated with the disease, OR <1 implies that genotype is protective. All *p*-values are two-tailed. In all statistical analyses, a two-tailed *p*-value of 0.05 or less was considered to indicate statistical significance.

Results

At 12 months after chemotherapy completion 114 patients with AIHF who had LVEF decline by 24.3% from the values before chemotherapy initiation (from 66.0 [61; 70] to 50 [47; 52]%) and NT-proBNP increase by 82.9% (from 55.25 [48.1; 64] to 323.0 [260.7; 377.7] pg/mL) were enrolled. At baseline women were of New York Heart Association (NYHA) class I (42.2%), class II (43.9%), and class III (7.9%). The control group comprised the women (n = 70) who received doxorubicin as part of chemotherapy for breast cancer, but did not develop AIHF (LVEF of 66 [64; 69]%). The Table 1 shows baseline demographic and clinical characteristics of patients depending on AIHF presence. Patients with AIHF had LV remodeling and higher values of NT-proBNP, and heart rate ($p = 0.025$). The gene polymorphisms of HIF1A gene (1772C>T, rs11549465) did not differ between patients.

All patients with AIHF were prescribed the optimal medical treatment. After 24 months of follow-up patients with AIHF had breast cancer remission and were divided into two groups: group 1 comprised women with adverse course of AIHF (n = 39), group 2 comprised those without it (n = 75). The baseline parameters did not differ (Table 2).

After 24 months of follow-up in group 1 the LVEF significantly ($p <0.001$) decreased by 13.5% from 52 (49; 54) to 49 (46; 51)%, end-systolic dimension increased by 5.9% ($p = 0.012$), end-diastolic dimension by 7.9% ($p = 0.034$), and 6-minute walk test distance decreased by 20% ($p = 0.017$); there was a tendency in the NT-proBNP levels decrease by 7% ($p = 0.087$). In group 2 the LVEF improved by 9.3% ($p = 0.032$), and the levels of NT-proBNP decreased ($p <0.001$) by 30.6% (Table 3).

The presence of C/T genotype of HIF1A gene (1772C>T, rs11549465) (OR = 3.65; $p = 0.009$) was related to the adverse course of AIHF. The C/C genotype of HIF1A gene (1772C>T, rs11549465) (OR = 0.123; $p = 0.001$) was significantly associated with the favorable course of AIHF.

Dynamics of echocardiographic parameters, NT-proBNP levels and 6-minute walk test distance during follow-up period depending on gene polymorphisms of HIF1A gene (1772C>T, rs11549465) (Table 4). Women with C/T genotype of HIF1A gene (1772C>T, rs11549465) had further progression of AIHF. In these patients LVEF significantly ($p <0.001$) decreased by 11.8% from 51 (47; 53) to 45 (43; 46)%, end-systolic dimension increased by 7.8% ($p <0.001$) and end-diastolic dimension by 5.2% ($p <0.001$). Carriers of T/T genotypes did not have significant changes in these parameters with the exception of NT-proBNP levels which significantly decreased during follow-up period ($p <0.05$). In patients with the C/C genotype LVEF increased by 4% ($p = 0.049$), and NT-proBNP levels decreased by 37.2% ($p <0.001$).

Discussion

We demonstrated here for the first time that the presence of C/T genotype of the hypoxia-inducible factor 1alpha gene (1772C>T, rs11549465) in women without previous CVD was related to the adverse course of AIHF during 24 months.

Heart failure is a major complication in cancer treatment due to the cardiotoxic effects of anticancer drugs, especially from the anthracyclines such as DOX.[2] Most patients remain asymptomatic during and after drug in-

Table 1 – Baseline demographic and clinical characteristics of patients depending on AIHF presence

Characteristics	Patients with AIHF n = 114	Patients without AIHF n = 70	*p*-value
Age (years)	48 (46; 52)	45 (42; 50)	0.057
CD of doxorubicin, mg/m^2	360 (300–360)	360 (300–360)	0.818
Chemotherapy regimen, n (%) AC TAC	 61 (53.5) 53 (46.5)	 39 (55.7) 31 (44.3)	 0.631 0.654
Breast cancer stage 2A–2B, n (%)	72 (63.2)	44 (62.9)	0.467
Breast cancer stage 3A–3B, n (%)	42 (36.8)	26 (37.1)	0.972
LVEF, %	50.0 (47; 50)	66.0 (64; 69)	<0.001
End-diastolic dimension, mm	49 (46; 51.0)	44.0 (42; 47)	<0.001
End-systolic dimension, mm	36 (33; 38)	31 (29; 33)	<0.001
Body mass index	23.7 (21.4; 26.2)	23.6 (21.9; 25.7)	0.859
Heart rate, bpm	84 (78; 93)	75 (69; 81)	0.025
Systolic blood pressure, mmHg	115 (110; 120)	115 (110; 120)	0.744
Diastolic blood pressure, mmHg	70 (70; 80)	70 (70; 80)	0.932
Chronic obstructive lung disease	13 (11.4)	9 (12.8)	0.747
eGFR, mL/min/m^2	89 (78; 96)	88 (76; 98)	0.876
6-minute walk test distance, m	419 (358; 467)	567 (563; 577)	<0.001
Total cholesterol, mmol/L	5.2 (4.85; 5.7)	5.25 (4.8; 5.7)	0.882
Hemoglobin, g/L	109.5 (100; 117)	109.5 (99; 117.5)	0.798
NT-proBNP, pg/mL	323.0 (260.7; 377.7)	54.65 (45.7; 72.6)	<0.001
HIF1A gene (1772**C**>T, rs11549465) C/C C/T T/T	 72 (63.2) 36 (31.5) 6 (5.3)	 45 (64.3) 23 (32.6) 2 (3.1)	 0.972 0.857 0.599

AC-regimen – combination of doxorubicin and cyclophosphamide; BMI – body mass index (calculated as weight in kilograms divided by height in meters squared); CD – cumulative dose; eGFR – estimated glomerular filtration rate (CKD-EPI); HIF1A – hypoxia-inducible factor 1alpha; LA – left atrium; TAC-regimen – combination of doxorubicin, cyclophosphamide, and docetaxel.

Table 2 – Baseline demographic and clinical characteristics of patients depending on course of AIHF

Characteristics	Group 1, n = 39	Group 2, n = 75	*p*-value
Age (years)	48 (46; 50)	48 (45; 50)	0.067
CD of doxorubicin, mg/m^2	360 (300–360)	360 (300–360)	0.952
Chemotherapy regimen, n (%) AC TAC	 25 (64.1) 14 (35.9)	 39 (52.0) 36 (48.0)	 0.104 0.102
Functional class of HF (NYHA) I functional class II functional class III functional class	 30 (76.9) 7 (17.9) 2 (5.2)	 43 (57.3) 25 (31.6) 7 (9.3)	 0.187 0.073 0.321
Breast cancer stage 2A–2B, n (%)	23 (59.0)	49 (65.3)	0.967
Breast cancer stage 3A–3B, n (%)	16 (41.0)	26 (34.7)	0.972
LVEF, %	52.0 (49; 54)	49 (46; 51)	0.199
LA, mm	30 (29; 32)	30 (29,5; 33.3)	0.871
End-diastolic dimension, mm	48 (46; 51.0)	49.0 (46; 51)	0.617
End-systolic dimension, mm	35 (33; 38)	36 (33; 39)	0.604
Body mass index	24.5 (21.5; 26.2)	23.5 (21.2; 25.5)	0.414
Heart rate, bpm	81 (68; 87)	75 (69; 81)	0.025
Systolic blood pressure, mmHg	115 (110; 120)	115 (110; 120)	0.082

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Table 2 – Baseline demographic and clinical characteristics of patients depending on course of AIHF *(Dokončeni)*

Characteristics	Group 1, n = 39	Group 2, n = 75	*p*-value
Diastolic blood pressure, mmHg	70 (70; 80)	70 (70; 80)	0.297
Current smoker, n (%)	6 (15.4)	11 (14.7)	0.654
Chronic obstructive lung disease	4 (10.3)	9 (12.0)	0.937
Childbearing potential women, n (%)	14 (35.9)	29 (38.7)	0.876
Menopause, n (%)	25 (64.1)	46 (61.3)	0.989
eGFR, mL/min/m^2	81.5 (73; 97)	83 (77; 98)	0.523
6-minute walk test distance, m	412 (364; 466)	399 (348; 457)	0.617
Total cholesterol, mmol/L	5.0 (4.5; 5.4)	5.1 (4.3; 5.5)	0.412
Hemoglobin, g/L	107 (98; 113)	105.5 (99.5; 118)	0.568
NT-proBNP, pg/mL	324.5 (265.4; 369.7)	318.9 (258.0; 381.8)	0.971
HIF1A gene (1772**C**>T, rs11549465) C/C C/T T/T	 15 (38.5) 22 (56.4) 2 (5.1)	 57 (76.0) 14 (18.7) 4 (5.3)	 <0.001 0.016 0.902

AC-regimen – combination of doxorubicin and cyclophosphamide; BMI – body mass index (calculated as weight in kilograms divided by height in meters squared); CD – cumulative dose; eGFR – estimated glomerular filtration rate (CKD-EPI); HF – heart failure; HIF1A – hypoxia-inducible factor 1alpha; LA – left atrium; MMP – matrix metalloproteinase; TAC-regimen – combination of doxorubicin, cyclophosphamide, and docetaxel.

Table 3 – Dynamics of echocardiographic parameters during follow-up period

Parameter	Baseline		*p*-value	At 24 months		*p*-value
	Group 1 (n = 39)	Group 2 (n = 75)		Group 1 (n = 39)	Group 2 (n = 75)	
LVEF, %	52.0 (49; 54)	49 (46; 51)	0.199	45 (44; 49)#	54 (49; 55)#	<0.001
LA, mm	30 (29; 32)	31 (28; 33)	0.431	33 (30; 33)#	29 (27; 31)	<0.001
EDD, mm	48 (46; 51.0)	49.0 (46; 51)	0.617	51 (48; 52)#	48 (46; 49)	<0.001
ESD, mm	35 (33; 38)	36 (33; 39)	0.604	38 (36; 39)#	35 (32; 37)	<0.001
NT-proBNP, pg/mL	324.5 (265.4; 369.7)	318.9 (258.0; 381.8)	0.971	301.7 (271.6; 343.1)	221.3 (190.1; 272.5)#	<0.001
6-MWT distance, m	412 (364; 466)	399 (348; 457)	0.617	329.9 (310.8; 404.2)#	429 (391; 454)	0.001

6-MWT – 6-minute walk test; EDD – end-diastolic dimension; ESD – end-systolic dimension; LA – left atrium; LVEF – left ventricle ejection fraction; NT-proBNP – N-terminal pro-B-type brain natriuretic peptide; # - statistically significant in comparison with baseline levels.

fusion, however, LV dysfunction and HF can occur up to decades after exposure,[1] and HF induced by anthracyclines is often resistant to therapy and has a mortality rate of up to 79%.[1] For this reason, cardiologists, oncologists, and basic scientists are combining their efforts in order to better evaluate the mechanisms of its development and progression.

Hypoxia inducible factors are proteins that rapidly accumulate during hypoxia and manage transcriptional control over various target genes.[5] Hypoxia-inducible factor-1a and HIF2A control cellular adaptation to both acute and chronic hypoxia, and these isoforms can be involved in the redox state and on proinflammatory pathways.[6] During commonly encountered physiological states, a decrease in environmental oxygen enhances signaling through both HIF1A and HIF2A. However, under the pathological conditions that occur in chronic cardiac, renal, and vascular diseases, HIF signaling is also stimulated by abnormalities in mitochondria and peroxisomes, the most important oxygen-consuming organelles in cells. Derangements in these cellular constituents redirect the use of oxygen toward the generation of reactive oxygen species (ROS), which inhibit prolyl hydroxylases.[12] The activation of HIFs by oxidative stress is dependent on the presence of mitochondria that are able to consume oxygen and generate ROS.[8,12] Once activated by oxygen-related organelle stresses, HIF1A and HIFHIF1A2A act to reduce these stresses by decreasing the amount of oxygen consumed by mitochondria and peroxisomes.[13] HIF1A directly inhibits both the biogenesis and oxidative functions of mitochondria.[14] In addition, both HIF1A and HIF2A promote autophagy, a lysosome-dependent degradative pathway that mediates the clearance of dysfunctional organelles. HIF1A enhances the autophagic clearance of damaged mitochondria,[15] whereas HIF2A stimulates the autophagic disposal of injured peroxisomes[16] that all lead to the development of mitochondrial dysfunction.

Table 4 – Dynamics of echocardiographic parameters, NT-proBNP levels, and 6-minute walk test distance during follow-up period depending on gene polymorphisms of HIF1A gene (1772C>T, rs11549465)

Parameter	Baseline			*p*-value	At 24 months			*p*-value
	HIF1A gene (1772C>T, rs11549465)				HIF1A gene (1772C>T, rs11549465)			
	C/C (n=72)	C/T (n=36)	T/T (n=6)		C/C (n = 73)	C/T (n = 36)	T/T (n = 5)	
LVEF, %	50 (47; 52)	51 (47; 53)	49 (47; 50)	0.626	52 (46; 55)#	45 (43; 46)#	50 (45; 52.5)	<0.001
LA, mm	30 (29; 33)	31 (28; 33)	31 (29; 32)	0.431	29 (28; 31)	33 (30; 34.5)#	30 (29; 33)	<0.001
EDD, mm	49 (46; 51)	48.5 (46.0; 51.0)	48 (46; 50)	0.959	48 (46; 55)	52.6 (49; 52)#	49 (46; 50)	<0.001
ESD, mm	36 (33; 38)	37 (34; 39)	37 (35; 38)	0.399	36 (33; 37)	39 (39; 40)#	36 (34; 38)	<0.001
NT-proBNP, pg/mL	315.9 (245.6; 374.5)	343.5 (279.8; 377.6)	349.0 (279.0; 383.2)	0.652	230.3 (201.0; 288.1)#	330.3 (271.8; 343.6)	270.3 (223; 305.6)#	0.039
6-MWT distance, m	411 (352; 465)	415.5 (370; 479)	450 (444; 483)	0.176	451 (385; 447)#	387 (386; 429.0)#	458 (370.5; 447)	0.001

6-MWT – 6-minute walk test; EDD – end-diastolic dimension; ESD – end-systolic dimension; LA – left atrium; LVEF – left ventricle ejection fraction; NT-proBNP – N-terminal pro-B-type brain natriuretic peptide; # - statistically significant in comparison with baseline levels.

Anthracycline-induced cardiotoxicity is a complex multifactorial process. The main well-established mechanism is that anthracyclines can bind both DNA and topoisomerase-2 (Top2) in order to form the ternary Top2-DOX-DNA cleavage complex which triggers cell death and gain further toxicity upon conversion to reactive oxygen species (ROS), like superoxide anion and its dismutation product hydrogen per-oxide.[17] Besides inhibiting Top2α in proliferating cells, DOX can target Top2β, which is also the only known type 2 topoisomerase present in cardiac mitochondria.[10] Zhang et al. demonstrated that DOX treatment induces significant changes in the expression of genes controlling both mitochondrial structure and metabolisms,[9] as well as DOX, preferentially accumulates in the mitochondria of cardiomyocytes, strongly impacting both the structure and the activity of these organelles that cause the increased ROS production.[10] Anthracycline-mediated production of these reactive species in turn may potentially lead to imbalances in HIFs proteins and inhibiting of HIF1A/ARNT heterodimer to the target gene enhancer,[4] and, as a consequence, be one of the trigger mechanisms for the development of acute heart dysfunction induced by anthracyclines. However, it is not clear whether it affects the development and progression of HF in the long-term periods after chemotherapy treatment.

The 1772C> T (rs11549465) polymorphism in HIF1A gene causes the replacement of proline for serine in the oxygen-dependent degradation domain. A protein with such a substitution has increased transactivation ability under conditions of both hypoxia and normoxia.[18] It was established that expression of HIF1A gene is increased in the failing heart, both experimentally and clinically[19,20] and it is associated with a poor prognosis in patients with chronic and acute heart failure.[21,22] There is a growing body of evidence for impaired HIF-1 activation in diabetes,[23] coronary artery disease,[24] and obesity.[25] Endogenous HIF-1 target gene expression was also inhibited by anthracyclines in a dose-dependent manner.[18] However, it is unlikely that the activation of HIF1A is an initial mechanism in the development of late AIHF, since in our study the gene polymorphisms of HIF1A did not differ between patients with AIHF and without it. Most likely it is just a consequence of the metabolic continuum, which begins with mitochondrial damage, oxidative stress, the development of endothelial dysfunction, and the initiation of apoptosis[19] and, ultimately, stabilization of HIF1A at high levels occurs. All these processes lead to HIF complex bindings to hypoxia response elements at target gene loci, regulating expression of both the HIF1A gene and other genes involved in the hypoxic response on both a cellular and systemic levels.[21] Perhaps, HIF1A impairs mitochondrial biogenesis, further limiting the generation of adenosine triphosphate,[12] and promotes inflammation in cardiomyocytes,[19] causes the development of systemic endothelial dysfunction, and may activate the sympathetic nervous system in chronic heart failure.[26] And therefore, when all of the following processes are launched and the heart remodeling processes have already started, the direct activation of the HIF1A gene occurs; it already plays a crucial role in late AIHF progression.

Conclusions

Thus, the polymorphisms of hypoxia-inducible factor 1alpha gene (1772C>T, rs11549465) in women without previous CVD was associated with adverse course of AIHF during 24 months, and it may be recommended for women with AIHF without previous cardiovascular diseases in the risk assessment of AIHF progression.

Key points

HIFs are proteins that rapidly accumulate during acute and chronic hypoxia, control cellular adaptation and manage transcriptional control over various target genes. Expression of HIF1A gene is increased in the failing heart and it is associated with a poor prognosis in patients with chronic and acute HF. In anthracycline consume the activation of HIFs by oxidative stress is dependent on the presence of mitochondria that are able to consume oxygen and generate ROS. But it is not clear whether it affects the development and progression of HF in the long-term periods after chemotherapy treatment.

We demonstrated here for the first time that the presence of C/T genotype of the HIF1A gene (1772C>T, rs11549465) in women without previous CVD was related to the adverse course of AIHF during 24 months. Most likely that is just a consequence of the metabolic continuum, which begins with mitochondrial damage, oxidative stress, the development of endothelial dysfunction, and the initiation of apoptosis and, ultimately, stabilization of HIF1A at high levels occurs and HIF1A gene expressions at late stages of AIHF.

Future direction

The identification of new mechanisms for the development and progression of AIHF may be used to search for more promising methods of treatment. Perhaps, the use of trimetazidine or other drugs that can reduce hypoxia will help to improve the prognosis in patients at late stages of AIHF.

Limitations

The main limitation of the study was the small sample of patients and lack of data of HIF serum levels and other biomarkers. Further studies with bigger sample size are needed, it is also necessary to compare gene expression with serum levels of HIFs.

Author contributions

All authors provided important intellectual contribution to the work: KVK contributed to design, data collection, analysis, and drafted the first version of the manuscript; EVG and ATT supervised research work and contributed to conception, design and data interpretation; SNS and ENB contributed to drafting the manuscript, crafting the discussion, and revising for important intellectual content; MNN, AAP, and ETR contributed to conception of the work revised for important intellectual content. All authors carefully read the manuscript and gave final approval.

Conflicts of interest

The authors declare no conflict of interest.

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