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Changes in lipid metabolism in osteoarthritis patients with arterial hypertension, obesity and type 2 diabetes mellitus depending on matrilin-3 (RS77245812) and interleukin-10 (RS1800872) genes polymorphism

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Abstract
The changes of lipid metabolism were evaluated depending on genes polymorphism matrilin-3 (*MATN3*, rs77245812) and interleukin-10 (*IL*-10, rs1800872) in 74 osteoarthritis patients combined with AH, abdominal obesity, type 2 diabetes mellitus and 25 practically healthy persons. The *C908T* polymorphism of MATN3 gene associate with a high density level cholesterol (HDL) decrease (F=14.97), with increasing of low density level cholesterol (LDL) concentration (F=10.54) and increasing of an atherogenic index (AI) (F=25.12) without direct dependence on gene genotypes. While the *IL-10* gene (rs1800872) promoter site associates with an increased total cholesterol (TC) content (F=20.94), slightly less with Triglycerides (F=3.46), LDL (F=7.55), very low density level cholesterol (VLDL) (F=8.78) and reduced level of HDL (F=12.78), especially in the A allele carriers. Almost half of the examined patients (56.76%) had a mixed atherogenic IIb type of dyslipidemia by D. Fredrickson and every third (27.03%) patient evidently had an endogenous type IV hyperlipidemia (hypertriglyceridemia).

Keywords: MATN3 (rs77245812), *IL*-10 (rs1800872) genes, osteoarthritis, lipid metabolism

Introduction
Osteoarthritis (OA) is the most common disease of the joints and the leading cause of disability among the adult population. The basic pathogenetic changes during OA have been proven in many studies, including in particular, progressive loss of articular cartilage, cartilage calcification, osteophyte formation, remodeling of subchondral bone and inflammation of the synovial membrane [1-3]. At first, disturbances at the molecular level occur (abnormal metabolism in tissues of the joints) with subsequent anatomical and physiological disorders that lead to the development of a clinically disease apparent [3, 4].
It has been established that lipid metabolism disorders can contribute to the development of dystrophic changes in the vascular wall and articular cartilage, the progression of arterial hypertension (AH), OA and other components of the metabolic syndrome aggravating the course and quality of life of the patients [5-7]. Under conditions of dyslipidemia oxidized low density lipo-protein (LDL) reduces the activity of endothelial NO synthase (eNOS) and the bioavailability of NO [8-10]. In response to the deleterious effect of excess blood lipids, the endothelium reacts with increased synthesis of vasoconstrictors and insufficient synthesis of vasodilators. On the membrane of endothelial cells there are molecules of adhesion that provide penetration into the vascular wall of T-lymphocytes and macrophages that is, they support chronic local inflammation and contribute to the progression of OA [11].
However, the questions of molecular and genetic mechanisms' association with OA appearance and consequent metabolic disorders remain unsolved and require further research.

The aim of the research: To study changes in lipid metabolism in osteoarthritis patients combined with AH, abdominal obesity (AO) and type 2 diabetes mellitus (DM 2) depending on genes polymorphism matrilin-3 (*MATN3*, rs77245812) and interleukin-10 (*IL*-10, rs1800872).

Materials and methods

Compliance with bioethics

Study had been performing for 3 years (2015-2017 yy) in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled patients were treated in the Regional Clinical Hospital, Rheumatology Department (Chernivtsi, Ukraine). Genetic bench study performed at the laboratory of the State institution «Reference centre of molecular diagnostics of the Ministry of Health of Ukraine» (Kyiv). After screening (matching inclusion/ exclusion criteria) 74 patients with OA, AO, AH and type 2 DM were selected for further examination. The control group included 25 practically healthy individuals who were not relatives with the patients, without reliable differences of sex and age.

Diagnosis of Osteoarthritis, Arterial Hypertension, Abdominal Obesity and Type 2 Diabetes Mellitus

OA diagnosis was based upon complaints, medical history (anamnesis), the results of clinical, laboratory and instrumental examination according to diagnostic criteria of Ministry of Healthcare of Ukraine (2016) "Clinical Protocol of Providing Medical Care for OA Patients" and American College of Rheumatology (ACR, 1991) [11, 12].

All patients were examined comprehensively: general clinical, laboratory and instrumental examination according to recommendation. Anthropometric data were assessed by height, weight, waist and hips circumference measurement (WC, HC), body mass index (BMI) calculating. The obesity and abdominal obesity determined according to International criteria: WC >94 cm for men and WC>80 cm for women, BMI ≥30kg/m^2 [13].

The diagnosis of AH was verified in compliance with Ministry of Healthcare Order of Ukraine #384 dated 24.05.2012 and National Ukrainian (2012) and European (ESC, ESH, 2013) recommendations [14, 15]. DM diagnosis was verified in accordance to Ministry of Healthcare Order of Ukraine #1118 dated 21.12.2012 and based on "Unified Clinical Protocol of Specialized Medical Help: Type 2 DM" (2012).

Genotyping

The polymorphic variants of genes *MATN3 (C908T*, rs77245812) and *IL-10 (C-592A*, rs1800872) were studied by polymerase chain reaction (PCR) method using oligonucleotide primers of the company «Metabion» (Germany) according to the modified protocols [16, 17]. Amplification products of DNA fragments of genes were digested by hydrolysis using restriction enzyme *AflIII* ("New England BioLabs", Great Britain) and *RsaI* ("Thermo Scientific", USA) accordingly [2]. The resulting fragments were analysed in 3% agarose gel ("Cleaver Scientific", Great Britain) with the addition of ethidium bromide, molecular weight marker Gene Ruler 50 bp DNA Ladder ("Thermo Scientific", USA), and further visualization by using transilluminator and Vitran software.

Lipid profile metabolism investigation

The lipid profile in OA patients was studied by analyzing the content of total cholesterol (TC), low and very low density level cholesterol (LDL, VLDL cholesterol), and high density level cholesterol (HDL cholesterol), triglycerides, or triacylglycerol (TG) in plasma with the following calculation of the atherogenic index (AI): AI=(TC-HDL)/HDL [13-15]. The "target" AI for people under 30 years was considered to be <2.5 standard units (s.u.), for people older than 30 years it should be <3.5 s.u. The "target" levels of TC, LDL C, VLDL C and HDL C were verified according to national and international recommendation, depending on the patients' risk [13-15].

Statistical analysis

The statistical analysis was performed using MS® Excel® 2007™ and "Statistica 7.0" (SPSS). The reliability of data for independent samples was calculated according to t-test Student (with the distribution of ranges close to normal), or U-criterion Wilcoxon-Mann-Whitney (with uneven distribution). The analysis of qualitative features was performed according to the χ^2 criterion. The difference was considered reliable at $p<0.05$.

Results and Discussion

Patients' average age was 58,03 ± 14,91 years, and the disease duration was within the limits of 5-32 years (12,17 ± 8,83 years). The gender distribution was following: 78, 38% (58) females and 21,62% (16) males. The control group included 25 practically healthy individuals; representatives by age and gender.

The lipids content in the blood plasma of OA patients depending on the genes' genotypes *MATN3* (*C908T*) and *IL-10 (C-592A)* is shown in the tables 1 and 2. TC was higher than the population norm in almost 2/3 (63.51%) of patients (n=47), TG and LDL cholesterol – in 74.32% (n=55) subjects, high level of VLDL cholesterol and AI had every second patient (56.76%; n=42) and HDL cholesterol was contrary lower than the norm in 54.05% of patients (n=40). The main lipid metabolism indices showed the presence of the mixed atherogenic dyslipidemia IIb type (by D. Fredrickson) in the majority of examined patients (56.76%) and almost every third (27.03%) patient evidently had an endogenous type IV hyperlipidemia (hypertriglyceridemia).

Individuals with a higher level of TC were observed more frequently among the CC-genotype carriers of MATN3 gene by 25.72% ($p = 0.003$), TG and LDL cholesterol - by 48.58% ($p<0.001$) and AI - by 17.14% ($p = 0.043$) (Table 1).

Table 1: Production levels of lipid fractions in the blood of patients with osteoarthritis depending on the polymorphous variants of the *MATN3* (*C908T*) gene.

Index	Production levels, n	Gene *MATN3 g*enotypes, n (%)		χ^2 p
		***CC*, n=70**	***CT*, n=4**	
TC	Within the norm, n=27	26 (37.14)	1 (25.0)	χ^2<1.0 p>0.05
	Above the norm, n=47	44 (62.86)	3 (75.0)	
χ^2; p		χ^2=9.26 p=0.003	χ^2<1.0 p>0.05	-
TG	Within the norm, n=19	18 (25.71)	1 (25.0)	χ^2<1.0 p>0.05
	Above the norm, n=55	52 (74.29)	3 (75.0)	
χ^2; p		χ^2=33.03 p<0.001	χ^2<1.0 p>0.05	-
LDL C	Within the norm, n=19	18 (25.71)	1 (25.0)	χ^2<1.0 p>0.05
	Above the norm, n=55	52 (74.29)	3 (75.0)	
χ^2; p		χ^2=33.03 p<0,001	χ^2<1.0 p>0.05	-
HDL C	Within the norm, n=34	31 (44.29)	3 (75.0)	χ^2<1.0 p>0.05
	Below the norm, n=40	39 (55.71)	1 (25.0)	
χ^2; p		χ^2=1,83 p>0.05	χ^2<1.0 p>0.05	-
VLDL C	Within the norm, n=32	30 (42.86)	2 (50.0)	χ^2<1.0 p>0.05
	Above the norm, n=42	40 (57.14)	2 (50.0)	
χ^2; p		χ^2=2,86 p>0.05	p>0.05	
AI	Within the norm, n=32	29 (41.43)	3 (75.0)	χ^2<1.0 p>0.05
	Above the norm, n=42	41 (58.57)	1 (25.0)	
χ^2; p		χ^2=4.11 p=0.043	p>0.05	-

Note. TC – total cholesterol; HDL / LDL / VLDL C – high / low / very low density level cholesterol; TG – triacylglycerols; AI – atherogenic index.

The higher relative frequency of people with TC higher than the population norm was observed in the mutational *A*-allele carriers of the *IL-10 gene* by 56.96% and 64.1% (p<0.001) and among the *CC*-genotype carriers prevailed the ones with the normal TC plasma content – by 56.96% (p<0.001) (table 2). The others lipidogram indices didn't depend on the IL-10 gene polymorphous variants. However, among *C* allele carriers patients with the TG higher content were more often detected – by 64.10% (p<0.001) and 28.58% (p=0.032) and LDL cholesterol – by 33.34% and 64.28% (p<0.001) accordingly. Higher AI was more often identified in *AA*-genotype patients – by 71.42% (p=0.015) and among *A* allele carriers of the *IL-10 gene*. The HDL cholesterol was lower in the *CA* genotype subjects – by 21.42% (p=0.001) (table 2).

Table 2: Production levels of lipid fractions in the blood of osteoarthritis patients depending on the polymorphous variants of the *IL-10* (*C-592A*) gene.

Index	Production levels, n	Gene *IL-10* genotype, n (%)			χ^2 p
		***CC*, n=39**	***CA*, n=28**	***AA*, n=7**	
TC	Within the norm, n=27	25 (64.10)	2 (7.14)	0	χ^2=26.27 p<0.001
	Above the norm, n=47	14 (35.90)	26 (92.86)	7 (100.0)	
χ^2; p		χ^2=6.21 p=0.013	χ^2=41.14 p<0.001	-	-
TG	Within the norm, n=19	7 (17.95)	10 (35.71)	2 (28.57)	χ^2=2.73 p>0.05
	Above the norm, n=55	32 (82.05)	18 (64.29)	5 (71.43)	
χ^2; p		χ^2=32.05 p<0.001	χ^2=4.57 p=0.032	χ^2<1.0 p>0.05	-
LDL C	Within the norm, n=19	13 (33.33)	5 (17.86)	1 (14.29)	χ^2=2,57 p>0,05
	Above the norm, n=55	26 (66.67)	23 (82.14)	6 (85.71)	
χ^2; p		χ^2=8.67 p<0.001	χ^2=23.14 p<0.001	p=0.015	-
HDL C	Within the norm, n=34	21 (53.85)	11 (39.29)	2 (28.57)	χ^2=2.33 p>0.05
	Below the norm, n=40	18 (46.15)	17 (60.71)	5 (71.43)	
χ^2; p		χ^2<1.0 p>0.05	χ^2=20.78 p=0.001	χ^2<1.0 p>0.05	-
VLDL C	Within the norm, n=32	17 (43.59)	13 (46.43)	2 (28.57)	χ^2<1.0 p>0.05
	Above the norm, n=42	22 (56.41)	15 (53.57)	5 (71.43)	
χ^2; p		χ^2=1.28 p>0.05	χ^2<1.0 p>0.05	χ^2<1.0 p>0.05	
AI	Within the norm, n=32	20 (51.28)	11 (39.29)	1 (14.29)	χ^2=3.60 p>0.05
	Above the norm, n=42	19 (48.72)	17 (60.71)	6 (85.71)	
χ^2; p		χ^2<1.0 p>0.05	χ^2=2.57 p>0.05	p=0.015	-

Note. TC – total cholesterol; HDL / LDL / VLDL C – high / low / very low density level cholesterol; TG – triacylglycerols; AI – atherogenic index.

The relative number of OA patients with higher TC level and AI prevailed those with normal data by 26.75% (p=0.032), 21.71% (p=0.014) and 33.63% (p=0.048) accordingly without direct dependence on the polymorphic variants of *MATN3* (*C908T*) gene (table 3). No others reliable changes were detected depending on the above mentioned gene's genotypes.

The dispersion analysis confirmed the association of the MATN3 gene (rs77245812) promoter with a decrease in HDL cholesterol (F=14.97, p<0.001), an increase in the LDL cholesterol concentration (F=10.54, p=0.002), and an increase in AI (F=25.12, p<0.001) (table 3).

Table 3: The content of lipid fractions in patients with osteoarthritisdepending on genotypes of the *MATN3* (*C908T*) gene, M±m

Indices	Control group	Gene *MATN3* genotypes in patients	
		***CC*, n=70**	***CT*, n=4**
TC, mmol/l	4.56±0.33	5.78±0.44 p=0.032	5.55±0.20 p=0.014
TG, mmol/l	0.83±0.15	1.31±0.48	1.47±0.31
LDL C, mmol/l	2.83±0.24	3.27±0.35	3.02±0.37
HDL C, mmol/l	1.46±0.13	1.39±0.08	1.23±0.09
VLDL C, mmol/l	0.47±0.06	0.63±0.11	0.45±0.03
AI, s.u.	2.23±0.35	2.72±0.10	2.98±0.12 p=0.048

Notes. 1. TC – total cholesterol; HDL / LDL / VLDL C – high / low / very low density level cholesterol; TG – triacylglycerols, AI – atherogenic index. 2. p – the reliability of indexes difference in comparison with control group; p_{CC} – the differences in comparison with CC-genotype carriers of the *MATN3* gene.

The content of lipid fractions in the OA patients depending on the *IL-10* (*C-592A*) gene genotypes is shown in the table 4. The TC level was higher in OA patients regardless of polymorphic variants of *IL-10* gene by 22.37-40.35% (p≤0.029-0.008). Herewith, the content of the TG and LDL cholesterol in OA patients were higher, than in the control group but only in the homozygous mutational A allele carriers 2.04 times (p=0.003) and by 28.27% (p=0.054). Just the patients with the AA genotype had higher concentration of the VLDL cholesterol and AI, than the *C* allele and *CC* genotype carriers of the *IL-10* gene by 54.90% (p_{CC}=0.01), 49.06% (p_{CT}=0.015) and 13.82% (p_{CC}=0.028) accordingly and, on the contrary, the HDL cholesterol was lower by 14.08% (p_{CC}=0.031).

The dispersion analysis confirmed the association of the *IL-10* gene (rs1800872) promoter with an elevated TC level (F=20.94, p<0.001), TG (F=3.46, p=0.037), LDL C (F=7.55, p=0.001), VLDL C (F=8.78, p<0.001) and a decrease in HDL cholesterol (F=12.78, p<0.001) (table 4).

Table 4: The content of lipid fractions in patients with osteoarthritis depending on genotypes of the *IL-10* (*C-592A*) gene, M±m

Indices	Control group	Gene *IL-10* genotypes in patients		
		***CC*, n=39**	***CA*, n=28**	***AA*, n=7**
TC, mmol/l	4.56±0.33	5.58±0.31 p=0.029	5.78±0.29 p=0.008	6.40±0.41 p=0.0085
TG, mmol/l	0.83±0.15	1.26±0.32	1.32±0.52	1.69±0.11 p=0.003
LDL C, mmol/l	2.83±0.24	3.35±0.16	3.23±0.32	3.63±0.32 p=0.054
HDL C, mmol/l	1.46±0.13	1.42±0.09	1.38±0.11	1.22±0.07 p_{CC}=0.031
VLDL C, mmol/l	0.47±0.06	0.51±0.11	0.53±0.23	0.79±0.07 p=0.009 p_{CC}=0.01 p_{CA}=0.015
AI, s.u.	2.23±0.35	2.75±0.13	2.78±0.71	3.13±0.17 p.p_{CC}=0.028

Notes. 1. TC – total cholesterol; HDL / LDL / VLDL C – high / low / very low density level cholesterol; TG – triacylglycerols, AI – atherogenic index. 2. p – the reliability of indexes difference in comparison with control group; p_{CC} – the differences in comparison with CC-genotype carriers; p_{CA} – the reliability of indexes differences in comparison with CA-genotype carriers.

Discussions

Our results showed the presence of the mixed atherogenic IIb type of dyslipidemia by D. Fredrickson in the majority of examined OA patients (56.76%) and almost every third (27.03%) person evidently had an endogenous type IV hyperlipidemia (hypertriglyceridemia). In our opinion, the persistent chronic inflammatory reaction present during OA determines the development of moderately expressed pro-atherogenic changes in lipid metabolism which is enhanced by the presence of type 2 DM with typical manifestations for diabetic dyslipidemia: hypertriglyceridemia, moderate hypercholesterolemia, increased AI and a decrease in HDL cholesterol. At the same time, the TG and LDL cholesterol content were higher in the homozygous carriers of the mutational A allele of *IL-10* gene 2.04 times (p = 0.003) and by 28.27% (p = 0.054). Likewise the concentration of the VLDL cholesterol and AI - by 13.82-54.90% (p≤0.028-0.01) accordingly and on the contrary, the HDL cholesterol was lower by 14.08% (p_{CC}=0.031) which indicates that these patients suffer from more evident dyslipidemia. It was studied that the growth of LDL cholesterol due to OA leads to an increase in the synthesis of caveolin-1 which in turn inhibits of NO synthesis by inactivating endothelial NOS, whereas the deficiency of the latter potentiates the free radicals formation [7]. Thus, LDL cholesterol and their oxidized forms activate a cascade of pathophysiological reactions at the cellular level with subsequent damage to the target organs and the development of cardiovascular complications, since LDL cholesterol is able to reduce the sensitivity of the vascular receptors to the action of antihypertensive agents [18].

In other research was proven a connection between the LDL cholesterol levels and the brachial artery diameter growth values (r=0.543; p<0.05) in AH patients [19]. Angiotensin II is a major antagonist of NO and not only inhibits its synthesis but also converts already synthesized NO into toxic peroxynitrite which destroys endothelial cells and oxidizes LDL, which become harmful to the body. Oxidized LDLs stimulate the mineralization of the vascular wall and through the vascular endothelium cells induce expression of the monocytic chemotactic factor and macrophage colony promoting factor which in turn are stimulators of differentiation and ripening of osteoclasts. Oxidized LDLs can potentially stimulate osteoclast-mediated bone tissue resorption and osteoporosis and, at the same time, promote ectopic calcification of the vascular wall [19].

Angiotensin II also increases expression on endothelial cell receptors to LDL and LDL-uptake [20]. These processes activate the chronic immune inflammation and contribute to the development and progression of a number of systemic pathologies: hypertension, atherosclerosis, coronary heart disease including myocardial infarction, cerebral strokes, etc. [21, 22]. Under the influence of oxidized LDL the adhesion of leukocytes to the endothelium increases, monocytes' synthesis induces pro inflammatory interleukin-1 as well as a large number of growth factors, cytokines, and TNF-α are being expressed which also contributes to increased blood pressure, disturbed microcirculation including in joints [23].

Conclusions

1. The majority of examined patients (56.76%) had the evidence of the mixed atherogenic IIb type dyslipidemia by D. Fredrickson. Almost every third (27.03%) person evidently had an endogenous type IV hyperlipidemia (hypertriglyceridemia).
2. The MATN3 gene (rs77245812) polymorphism associates with a HDL cholesterol decrease (F = 14.97), LDL cholesterol concentration increase (F =10.54) and AI increase (F = 25.12) without the direct dependence on the gene genotypes. While the *IL-10* gene (rs1800872) promoter site associates with an increased content of TC (F = 20.94), slightly less with TG (F = 3.46), LDL cholesterol (F = 7.55), VLDL cholesterol (F = 8.78) and reduced level of HDL cholesterol (F = 12.78), especially in the A allele carriers.

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