Особенности липидома у больных с различной клинической вероятностью семейной гиперлипидемии

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Разработка современных методов оценки метаболома, таких как хромато-масс-спектрометрия, позволяет существенно расширить представления о липидном обмене в конкретных клинических ситуациях. Целью исследования было изучение особенностей липидома у больных с различной вероятностью семейной гиперлипидемией (СГХС). В исследовании приняли участие 35 пациентов — 15 мужчин (42,9%) и 20 женщин (57,1%). У 10 пациентов вероятность СГХС оценивали как низкую (1–2 балла), у 22 пациентов диагноз расценивали как вероятную СГХС (3–5 баллов). У 3 пациентов с определенной/вероятной СГХС имелись достоверно более высокий уровень сфингозина по сравнению с пациентами с низкой клинической вероятностью СГХС (144,36 ± 107,863 и 50,14 ± 62,409 нг/мл; р = 0,001). В случае семейной СГХС отмечали увеличение доли длинноцепочечного сфингомиелина SM 18 : 1/22 : 0 в соотношении с низкой вероятностью семейной СГХС (144,36 ± 107,863 и 50,14 ± 62,409 нг/мл; р = 0,001). В случае семейной СГХС также наблюдалось увеличение уровня галактозилцерамида SM 18 : 1/22 : 0 наряду с обратными корреляциями уровня сфингозина. Таким образом, у пациентов с высокой клинической вероятностью СГХС были выявлены изменения липидома, являющиеся маркерами риска сердечно-сосудистых осложнений.

Ключевые слова: атеросклероз, семейная гиперлипидемия, сфингомиелин, сфингозин, церамиды, маркеры риска

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ORIGINAL RESEARCH | LIPIDOLOGY

LIPIDOME FEATURES IN PATIENTS WITH DIFFERENT PROBABILITY OF FAMILY HYPERCHOLESTEROLEMIA

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Development of modern methods for metabolome assessment, such as gas chromatography–mass spectrometry, allows one to expand the knowledge about the features of lipid metabolism in various clinical conditions. The study was aimed to investigate lipidome features in patients with different probability of family hypercholesterolemia (FH). The study involved 35 patients: 15 men (42.9%) and 20 women (57.1%) with dislipidemia or early cardiovascular diseases which manifested below 55 in men and 60 in women (average age of patients was 49.8 ± 9.96). The family dislipidemia probability was evaluated using the Dutch Lipid Clinic Network Score. In 10 patients the probability of FH was low (score 1–2), 22 patients had possible FH (score 3–5). Three patients had probable or definite FH (score 6 in 2 patients, score 9 in one patient). Determination of molecular species of sphingomyelins, ceramides and sphingoid bases (sphingosine, sphinganine) as well as galactosylceramide was carried out using gas chromatography–mass spectrometry. In patients with definite/probable FH the sphinganine level was significantly higher compared with patients having low probability of FH (144.36 ± 107.863 and 50.14 ± 62.409 ng/ml; р = 0.01). In patients with FH, an increase in the proportion of long chain sphingomyelin SM 18 : 1/22 : 0 as well as a significant increase in the level of long chain ceramides with C 20 : 1 and C 22 : 1 was determined. Positive correlation of low-density lipoproteins and sphingosine level (р = 0.344; р = 0.047) together with negative correlation of high-density lipoproteins (HDL) and sphinganine level (р = –0.52; р = 0.002), and galactosylceramide level (р = –0.56; р = 0.001) were detected. Thus, in patients with high probability of FH the lipidome changes were observed, which could be considered the cardiovascular risk markers.

Keywords: atherosclerosis, family hyperlipidemia, sphingomyelins, sphingosine, ceramides, risk marker

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Compliance with ethical standards: the study was approved by the Local Ethics Committee of City Clinical Hospital № 51 (protocol № 02/19 dated February 7, 2019). Informed consent was obtained from all study participants.

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Lipid metabolism disorders, including the hereditary ones, are a key risk factor for atherosclerosis and its complications. The development of newest metabolome investigation methods, such as gas chromatography-mass spectrometry, allows one to expand the knowledge about the features of lipid metabolism in various clinical situations.

It was found that sphingolipids (sphingomyelins, ceramides, sphingosine, sphinganine, sphingosine-1-phosphate (S1P) etc.) can play a significant role [1]. A change in the ratio of various sphingolipids is detected in patients affected with certain metabolic, genetic and autoimmune diseases (Fabry disease, Niemann–Pick diseases, Gaucher disease etc., some types of epilepsy, migraine, Alzheimer’s disease).

An active study of the lipidome features associated with cardiovascular diseases is currently carried out. The prognostic value of some lipid fractions, mainly ceramides, in acute coronary syndrome has been revealed. The ratios of ceramides С 16 : 0, С 20 : 0, С 24 : 1 and their relationship to С 24 : 0 are considered as possible risk markers.

The prognostic value of ceramides was evaluated in prospective studies. The ceramides’ level was determined in patients with acute coronary syndrome [2]. It was found that the level of sphingomyelins, sphingosine, sphinganine-1-phosphate and ceramides can differ significantly in patients with acute and chronic forms of coronary heart disease [3].

At the same time, the lipidome features in patients with hereditary dislipidemia have not been studied. There are still no convincing data on the dynamics of the level of sphingolipids and ceramides against the background of lipid-lowering therapy. There are only single cases of comparison of the level of sphingolipids in patients without therapy and in patients receiving the lipid-lowering therapy [4, 5].

The study was aimed to investigate the features of sphingolipids in patients with different probability of family hypercholesterolemia.

**METHODS**

The study was carried out at the City Clinical Hospital № 51 in March-October 2019. Thirty five patients were surveyed. In the group of patients under study there were 15 men (42.9%) and 20 women (57.1%). Average age was 49.8 ± 9.96. Inclusion criteria: early manifestations of atherosclerosis (coronary heart disease, peripheral artery disease or cerebrovascular disease with the age of onset below 55 in men and 60 in women, and/or dislipidemia (LDL > 4,9 mmol/l). Exclusion criteria:

| Parameters               | Unlikely FH (n = 10) | Probable FH (n = 22) | Possible/definite FH (n = 3) | P     |
|--------------------------|----------------------|----------------------|-----------------------------|-------|
| TC, µmol/l               | 6.79 ± 0.627         | 8.04 ± 1.746         | 12.00 ± 5.344               | 0.006 |
| LDL, µmol/l              | 4.32 ± 0.4535        | 5.40 ± 0.973         | 7.24 ± 1.447                | 0.001 |
| HDL, µmol/l              | 1.52 ± 0.431         | 1.45 ± 0.457         | 1.15 ± 0.578                | 0.713 |
| TGL, µmol/l              | 1.81 ± 1.123         | 2.46 ± 3.245         | 5.80 ± 6.141                | 0.240 |
| Sphingosine, ng/ml       | 50.14 ± 62.409       | 83.59 ± 70.774       | 144.36 ± 107.863            | 0.051 |
| Sphinganine, ng/ml       | 0.752 ± 0.3713       | 0.895 ± 0.5841       | 1.663 ± 1.4619              | 0.142 |
| Galactosylceramide, ng/ml| 55.48 ± 29.867       | 66.60 ± 43.291       | 76.95 ± 25.626              | 0.473 |

**Sphingomyelins**

| SM 18 : 1/16 : 0, µg/ml | 18997.6 ± 13203.93 | 15407.2 ± 7769.07 | 9557.6 ± 2435.11 | 0.274 |
| SM 18 : 1/16 : 1, µg/ml | 1893.9 ± 714.16    | 1861.1 ± 1642.95  | 2208.3 ± 1071.19  | 0.432 |
| SM 18 : 1/18 : 0, µg/ml | 3848.2 ± 2447.91   | 3322.6 ± 1981.05  | 2392.6 ± 1758.81  | 0.629 |
| SM 18 : 1/18 : 1, µg/ml | 6138.8 ± 4915.11   | 5605.4 ± 2747.14  | 7240.6 ± 3716.52  | 0.806 |
| SM 18 : 1/20 : 0, µg/ml | 19573.6 ± 9198.49  | 22693.9 ± 15985.31| 24874.3 ± 6191.24 | 0.525 |
| SM 18 : 1/20 : 1, µg/ml | 55331.1 ± 34643.17 | 55612.7 ± 32720.49| 45554.0 ± 17549.55| 0.924 |

**Ceramides**

| C 18 : 0, µg/ml           | 3.70 ± 8.820        | 6.04 ± 9.740        | 0.016 ± 0.186        | 0.513 |
| C 20 : 0, µg/ml           | 224.70 ± 655.577    | 240.60 ± 431.668    | 367.67 ± 144.417    | 0.075 |
| C 20 : 1, µg/ml           | 85.10 ± 124.969     | 98.00 ± 229.133     | 698.67 ± 1138.155   | 0.019 |
| C 22 : 0, µg/ml           | 149.60 ± 347.728    | 75.96 ± 71.642      | 221.33 ± 170.365    | 0.100 |
| C 22 : 1, µg/ml           | 77.00 ± 82.254      | 60.80 ± 111.859     | 714.67 ± 1118.787   | 0.003 |
| C 24 : 0, µg/ml           | 587.80 ± 200.069    | 737.96 ± 354.259    | 782.00 ± 357.669    | 0.598 |
| C 24 : 1, µg/ml           | 206.20 ± 77.150     | 313.08 ± 254.952    | 465.67 ± 457.362    | 0.546 |
| C 18 : 0/C 24 : 0, µg/ml  | 0.0043 ± 0.00938    | 0.0079 ± 0.01275    | 0.0000 ± 0.00000    | 0.963 |
| C 24 : 1/C 24 : 0, µg/ml  | 0.3678 ± 0.13805    | 0.4600 ± 0.35776    | 0.5124 ± 0.29631    | 0.675 |

*Note: Kruskal–Wallis test.*
acute myocardial infarction, acute stroke, diabetes mellitus,
secondary dislipidemia. The study did not include patients who
received lipid-lowering therapy at the time of the examination.

In the beginning of the study 16 patients had arterial
hypertension (45.7%), 10 patients had coronary heart disease
(28.6%) and one patient had peripheral artery disease (2.9%).
Nineteen patients (54.3%) had significant family history of
cardiovascular diseases. Nine patients (25.7%) smoked in their
past but stopped smoking before inclusion in the study, 8 patients
smoked at the moment of inclusion in the study (22.9%).

The familial hypercholesterolemia (FH) probability was
evaluated using the Dutch Lipid Clinic Network Score. In 10
patients the probability of FH was low (score 1–2), 22 patients
had possible FH (score 3–5). Three patients had probable or
definite FH (score 6 in 2 patients, score 9 in one patient).

Blood sampling for biochemical analysis and mass
spectrometry was performed on the day the patients were
included in the study (in the morning on an empty stomach, after
a 12-hour fast). Blood was taken from the cubital vein into sterile
Vakutainer tubes. Serum was obtained by blood centrifuging
at a speed of 3000 rpm for 15 minutes. Parameters with the
following reference values were defined: total cholesterol (TC,
2.0–5.2 mmol/l), low-density lipoprotein cholesterol (LDL-C,
up to 3.3 mmol/l), high-density lipoprotein cholesterol (HDL-C,
0.91–1.56 mmol/l), blood serum triglycerides (TG, 0.50–1.70
mmol/l). To determine the parameters of serum, the CLIMA
MC-15 biochemical analyzer was used (RAL; Spain).

Lipids were extracted from plasma in accordance with
Bligh and Dyer Procedure [6]. Mass-spectrometry of molecular
species of sphingomyelins, ceramides and sphingoid bases
(sphingosine and sphinganine), as well as galactosylceramides,
was performed using the TSQ Endura Triple Quadrupole Mass
Spectrometer (Thermo Fisher Scientific; Germany) working
in the ММР mode. The pressure at the collision cell was 1.5
mTorr. The resolution on Q1 and Q3 was 1.2 Da.

Ceramides: fragmentation of the protonated and dehydrated
molecules was carried out at the energy of 25 eV down to ion
with m/z 264.4 Da, the dwell time was 25 ms.

Table 2. Blood lipids and sphingolipids in patients with family history and in patients without family history

| Parameters                  | No family history (n = 16) | Family history (n = 19) | p   |
|-----------------------------|---------------------------|------------------------|-----|
| TC, µmol/l                  | 8.11 ± 1.142              | 7.35 ± 1.881           | 0.026 |
| LDL, µmol/l                 | 5.49 ± 1.063              | 4.75 ± 0.820           | 0.039 |
| HDL, µmol/l                 | 1.63 ± 0.352              | 1.32 ± 0.479           | 0.034 |
| TG, µmol/l                  | 1.91 ± 1.119              | 2.57 ± 3.712           | 0.845 |
| Sphingosine, ng/ml          | 65.31 ± 55.298            | 82.37 ± 84.841         | 0.021 |
| Sphinganine, ng/ml          | 0.25 ± 0.447              | 0.47 ± 0.612           | 0.062 |
| Galactosylceramide, ng/ml   | 59.38 ± 46.989            | 67.00 ± 33.579         | 0.123 |

Sphingomyelins

| SM 18 : 1/16 : 0, µg/ml     | 15812.5 ± 8874.74         | 16142.37 ± 10210.772  | 0.678 |
| SM 18 : 1/16 : 1, µg/ml     | 1703.38 ± 1153.149        | 2079.74 ± 1637.244    | 0.635 |
| SM 18 : 1/18 : 0, µg/ml     | 3446.00 ± 2012.195        | 3115.37 ± 1651.906    | 0.942 |
| SM 18 : 1/18 : 1, µg/ml     | 6066.06 ± 4210.684        | 5388.68 ± 2755.342    | 0.862 |
| SM 18 : 1/20 : 0, µg/ml     | 21605.00 ± 6986.063       | 22197.89 ± 18558.786  | 0.756 |
| SM 18 : 1/20 : 1, µg/ml     | 57836.31 ± 37448.414      | 51584.74 ± 29461.884  | 0.684 |
| SM 18 : 1/22 : 0, µg/ml     | 8366.13 ± 3752.568        | 8090.74 ± 2977.416    | 0.862 |
| SM 18 : 1/22 : 1, µg/ml     | 387.56 ± 108.836          | 441.26 ± 183.190      | 0.672 |
| SM 18 : 1/24 : 0, µg/ml     | 1809.31 ± 983.979         | 2218.1 ± 1409.971     | 0.584 |
| SM 18 : 1/24 : 1, µg/ml     | 5398.19 ± 2713.511        | 5094.32 ± 2995.497    | 0.682 |

Ceramides

| C 18 : 0, µg/ml             | 6.31 ± 10.163             | 4.58 ± 8.946          | 0.213 |
| C 20 : 0, µg/ml             | 78.56 ± 150.510           | 391.21 ± 629.556     | 0.021 |
| C 20 : 1, µg/ml             | 57.38 ± 108.836           | 121.32 ± 257.882     | 0.010 |
| C 22 : 0, µg/ml             | 60.19 ± 64.744            | 130.21 ± 252.256     | 0.040 |
| C 22 : 1, µg/ml             | 47.75 ± 52.003            | 77.16 ± 133.153      | 0.252 |
| C 24 : 0, µg/ml             | 726.69 ± 334.931          | 622.16 ± 255.175     | 0.572 |
| C 24 : 1, µg/ml             | 324.25 ± 264.550          | 247.05 ± 181.903     | 0.457 |
| C 18 : 0/C 24 : 0, µg/ml    | 0.0072 ± 0.01166          | 0.0067 ± 0.01235     | 0.323 |
| C 24 : 1/C 24 : 0, µg/ml    | 0.4730 ± 0.37832          | 0.4078 ± 0.24104     | 0.872 |

Note: Mann–Whitney test.
Sphingomyelines: fragmentation of the protonated molecules was performed at the energy of 25 eV down to ion with m/z 184.1 Da, the dwell time was 25 ms. Sphingosine and its deuterated standard (d7, Avanti; USA) fragmentation of the protonated molecules was carried out at the energy of 12.5 eV down to ions with m/z 264.4 and 259.3 Da respectively. The dwell time was 25 ms.

Sphinganine: fragmentation of the protonated molecule was performed at the energy of 12.5 eV down to ion with m/z 266.4 Da, the dwell time was 50 ms.

Galactosylceramide d18 : 1/18 : 0: [M + H]^+ ion with a mass of 728.5 Da.

The following parameters of the ionization source were used: heater temperature 300 °C, capillary temperature 340 °C, sheath gas flow 45 arb, auxiliary gas flow 13 arb, sweep gas flow 1 arb.

Sphingosine d7, sphinganine, sphingomyelin d18 : 1/16 : 0, sphingomyelin d18 : 1/18 : 0, ceramide d18 : 1/16 : 0, ceramide d18 : 1/18 : 1, ceramide d18 : 1/18 : 0, ceramide d18 : 1/24 : 1, ceramide d18 : 1/24 : 0 and galactosylceramide d18 : 1/18 : 0 (Avanti; USA) were used as standards.

Chromatography

Chromatography was performed using the Ultimate 3000 system (Thermo Fisher Scientific; Germany) and Eclipse Plus C8 column 3.0 × 150 mm (Agilent; USA), the particle size was 3.5 μm. The temperature was 50 °C, and the flow rate was 400 μl/min.

When determining sphingosine, ceramides and sphingomyelin, the following mobile phases were used: phase A, water + 0.1% (v.v.) formic acid, phase B, methanol + 0.1% (v.v.) formic acid (0.7 minutes 55% of phase B, 100 % of phase B up to 10th minute).

When determining sphingomyelines, the Sphingomelian Porcine Brain 86002P mixture (Avanti; USA) and sphingomyelines d18 : 1/16 : 0, d18 : 1/18 : 0 (Avanti; USA) were used for calibration. The sum of peak areas of the MRM transitions MH^+→ m/z 264.4 Da and (MH-H2O)^+→ m/z 264.4 Da.

When determining sphingomyelines, the Sphingomelian Porcine Brain 860062P mixture (Avanti; USA) and sphingomyelines d18 : 1/16 : 0, d18 : 1/18 : 0 (Avanti; USA) were used for calibration. The sum of peak areas of the MRM transitions MH^+→ m/z 184.1 Da was used for calculation.

The sphinganine d18 : 0 content was determined by internal calibration (the standard was D-erythro-sphingosine d7, Sigma; USA) using the sum of peak areas of the MRM transitions (m/z 264.4 → m/z 264.4 Da for non-deuterated and m/z 267.4 → m/z 259.3 Da for deuterated sphingosine).

The sphingosine d18 : 0 content was determined by external calibration (the standard was DL-erythro-dihidrosphingosine, Sigma; USA) using the sum of peak areas of the MRM transitions (m/z 302.4 → m/z 266 Da).

Statistical analysis

Statistical analysis was carried out using the SPSS software, version 23.0 (IBM; USA). Quantitative variables were presented as mean with standard deviation. All variables were checked for compliance with normal distribution using the Shapiro-Wilk test. The distribution of all quantitative variables was different from normal. The significance of differences for two independent samples was evaluated using the Mann–Whitney test, and for three of more samples using the Kruskal–Wallis test. The significance of correlations was determined using the Spearman rank correlation test. The differences were considered significant when p < 0.05.

RESULTS

Comparison of blood lipids and sphingolipids was carried out in groups of patients with different probability of family hyperlipidemia (Table 1).

In patients with FH, an increase in the proportion of long-chain sphingomyeline SM 18 : 1/22 : 0 was noted, as well as a significant increase in the level of long chain ceramides, C 20 : 1 and C 22 : 1. No significant differences of C 18 : 0/C 24 : 0 and C 24 : 1/C 24 : 0 ratios were revealed.

The sphinganine level increase compared with a group of patients having a low probability of FH (r = 0.344, p = 0.047).

Fig. 1. Correlation of LDL and blood sphingosine level

Fig. 2. Correlation of HDL and blood sphinganine level
Table 3. Correlation of blood lipids, sphingomyelins and ceramides levels

|                         | TC, µmol/l | TG, µmol/l | LDL-C, µmol/l | HDL-C, µmol/l |
|-------------------------|------------|------------|---------------|---------------|
| **SM 18 : 1/16 : 0, µg/ml** | r = 0.178 | -0.104 | 0.084 | 0.162 |
|                         | p = 0.307 | 0.564 | 0.636 | 0.391 |
| **SM 18 : 1/16 : 1, µg/ml** | r = 0.260 | -0.171 | 0.093 | 0.045 |
|                         | p = 0.132 | 0.341 | 0.602 | 0.813 |
| **SM 18 : 1/18 : 0, µg/ml** | r = 0.257 | -0.140 | 0.278 | 0.236 |
|                         | p = 0.135 | 0.439 | 0.111 | 0.210 |
| **SM 18 : 1/18 : 1, µg/ml** | r = -0.139 | -0.095 | -0.202 | 0.077 |
|                         | p = 0.426 | 0.597 | 0.251 | 0.686 |
| **SM 18 : 1/20 : 0, µg/ml** | r = 0.363* | -0.015 | 0.164 | -0.110 |
|                         | p = 0.032 | 0.934 | 0.297 | 0.561 |
| **SM 18 : 1/20 : 1, µg/ml** | r = -0.101 | 0.111 | -0.098 | 0.334 |
|                         | p = 0.562 | 0.540 | 0.581 | 0.072 |
| **SM 18 : 1/22 : 0, µg/ml** | r = -0.017 | -0.313 | -0.155 | 0.165 |
|                         | p = 0.924 | 0.076 | 0.383 | 0.382 |
| **SM 18 : 1/22 : 1, µg/ml** | r = 0.062 | 0.146 | 0.125 | -0.187 |
|                         | p = 0.642 | 0.419 | 0.481 | 0.321 |
| **SM 18 : 1/24 : 0, µg/ml** | r = 0.048 | -0.183 | 0.100 | -0.254 |
|                         | p = 0.782 | 0.307 | 0.572 | 0.175 |
| **SM 18 : 1/24 : 1 µg/ml** | r = 0.217 | -0.297 | 0.148 | 0.046 |
|                         | p = 0.210 | 0.094 | 0.403 | 0.809 |
| **C 18 : 0, µg/ml** | r = 0.104 | -0.041 | 0.105 | -0.104 |
|                         | p = 0.552 | 0.820 | 0.556 | 0.584 |
| **C 20 : 0, µg/ml** | r = 0.055 | 0.141 | -0.003 | -0.420* |
|                         | p = 0.752 | 0.433 | 0.987 | 0.021 |
| **C 20 : 1, µg/ml** | r = -0.177 | 0.447** | -0.425* | 0.525** |
|                         | p = 0.310 | 0.009 | 0.012 | 0.003 |
| **C 22 : 0, µg/ml** | r = 0.015 | 0.342 | 0.049 | -0.429* |
|                         | p = 0.902 | 0.052 | 0.783 | 0.018 |
| **C 22 : 1, µg/ml** | r = 0.070 | 0.051 | -0.094 | -0.168 |
|                         | p = 0.689 | 0.776 | 0.598 | 0.374 |
| **C 24 : 0, µg/ml** | r = 0.475** | 0.100 | 0.334 | 0.008 |
|                         | p = 0.004 | 0.579 | 0.054 | 0.965 |
| **C 24 : 1, µg/ml** | r = 0.558** | 0.005 | 0.471** | 0.296 |
|                         | p = 0.000 | 0.976 | 0.005 | 0.112 |

Note: r — Spearman rank correlation; * — p < 0.005; ** — p < 0.001.
We analyzed the relationship between the level of various lipids and sphingolipids and the presence of a significant family history in patients (Table 2). In patients with significant family history, the higher level of sphingosine and significantly higher level of ceramides C 20 : 0, C 20 : 1, C 22 : 0 were observed.

Positive correlation of LDL and sphingosine level was revealed (Fig. 1). In addition, it was possible to identify negative correlation of HDL and sphingosin (Fig. 2) and galactosylceramide levels ($r = -0.56; p = 0.001$). Correlation analysis of sphingomyelin fractions level and ceramides with classical lipid fractions is presented in Table 3. A positive correlation of the level of ceramides C 24 : 0 and C 24 : 1 with the TC and LDL level is noteworthy. For C 20 : 0 ceramide, a positive correlation with the HDL and TG level and a negative correlation with LDL level were revealed. Negative correlation of the C 22 : 0 ceramide level with the HDL level was determined.

We analyzed correlations between the level of classical lipids and sphingolipids in patients having a significant family history and in patients with no significant family history. It is noteworthy, that a positive correlation between the level of LDL-C and sphingosine, revealed in the whole group, was of greater strength in patients with a significant family history ($r = 0.536; p = 0.022$). In patients with no significant family history, there was a negative correlation between LDL-C and sphingosine levels ($r = -0.351; p = 0.048$) (Fig. 3).

**DISCUSSION**

There are few studies of the sphingomyelines’ and ceramides’ profile in patients with family hyperlipidemia. The animal model of family hyperlipidemia associated with LDL receptor gene mutations demonstrated the significant increase of total sphingomyeline and C 18 : 0 ceramide in homozygotes [7]. In our study we noted only the SM 18 : 1/22 : 0 fraction and C 20 : 1 ceramide increase.

It was shown that the level of ceramides is associated with other coronary heart disease risk factors (obesity and insulin resistance). It was believed that some ceramide fractions were able to stimulate the synthesis of pro-inflammatory cytokines (e.g., tumor necrosis factor) in case of increased consumption of saturated fat with food [8]. In patients after bariatric surgery, a decrease in the level of atherogenic sphingomyelines and ceramides was observed earlier and to a much greater extent than weight loss, which correlated with a decrease in coronary risks [9].

It was found that in oxidized LDL the content of total sphingomyelines and ceramides was significantly higher, which could be an evidence of the role of sphingolipids in destabilization of atherosclerotic plaque and coronary heart disease and other disorders’ complications manifestation [10]. Sphingosine causes aggregation of Cu$^+$ peroxide vesicles and accelerates LDL peroxidation, making them more atherogenic. Long chain ceramides can serve as catalysts for said process. Ceramides with chain length C 6, C 8, C 10 do not possess such activity. Sphinganine, on the opposite, blocks peroxidation processes [11]. In our study we noted the significant increase of sphingosine level in patients with definite family dislipidemia. There were no significant differences in the sphinganine level in patients with low and high probability of family hyperlipidemia.

ApoE gene polymorphism (2/3/4) is associated with the increase of ceramide pathogenic fractions which may be related with increased coronary heart disease risk in young people [12].

The value of sphingomyelines C 16 : 0, C 22 : 0, C 24 : 0, C 24 : 1 in the carotid atherosclerosis pathogenesis in HIV patients was shown. For the long chain ceramides C 22 and C 24 a positive correlation with the TC and LDL level was determined [13]. Positive correlation of sphingomyelines SM d16 : 0/28 : 5, SM d18 : 1/24 : 1 and SM d18 : 1/16 : 0 with the TC and LDL level was revealed in the animal model of dislipidemia (ApoE-deficient mice). The level of said fractions in animals with hyperlipidemia was elevated. Sphingolipides of such kind are considered pro-atherogenic [14]. There is evidence that oxidative stress and lipotoxicity are associated precisely with an increase in the level of long chain ceramides, which, for example, becomes apparent in patients with insulin resistance [15]. In our study the SM 18 : 1/22 : 0 sphingomyeline was increased in patients with definite/probable hyperlipidemia. Positive correlations of blood cholesterol with ceramides C 24 : 0, C 24 : 1 level were revealed.

Our study had a number of limitations: single site study, small sample size, lack of data from large studies on the epidemiological relationship between detected changes in lipid component and cardiovascular events (heart attacks, strokes, cardiovascular death).
CONCLUSION

 Patients with definite/probable FH demonstrate not only high level of TC and LDL, but also the high level of pro-atherogenic sphingosine, sphingomyelin SM 18 : 1/22 : 0, and long chain ceramides (C 20 : 1; C 22 : 1). The revealed lipidome features require further clarification of their clinical significance.

Lipidome changes may help to explain the mechanism of increasing the risk and early onset of atherosclerosis in said group of patients.

Positive correlation of sphingosine with the LDL level in patients with significant family history is the evidence of the importance of sphingosine as an additional risk factor associated with the family nature of the disease.

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