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FAMILIAL DYSBETALIPOPROTEINEMIA (TYPE III HYPERLIPOPROTEINEMIA)

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SUMMARY

The aim of this work was to describe a series of cases of familial dysbetalipoproteinaemia (FD) – a rare recessive disorder of lipid metabolism. The study included 18 patients of both sexes, mean age was 42.4 years. Quantitative determination of total cholesterol (TC) and triglycerides (TG) was carried out by a unified enzymatic method, high density lipoprotein (HDL) and low-density lipoprotein (LDL) – by a direct homogeneous method. The APOE gene rs7412 variant was determined by real-time polymerase chain reaction (PCR) using adjacent samples and by melting reaction products after PCR. The frequency of FD according to DNA analysis among 367 patients with different types of hyperlipidemia was 4.9%. CHD was detected in 27.8% of patients. Different types of xanthomas were detected in 22.2% of patients. When comparing the initial lipid profile of patients with FD and those in the control group, significantly higher levels of TC, TG, LDL-C and non-HDL-C were observed, while plasma HDL-C levels were significantly lower than in the control group. On lipid-lowering therapy (statin and/or fibrate), the average levels of TC, TG and non-HDL cholesterol decreased approximately 2 times from baseline ($p<0.002$), and LDL decreased 1.5 times ($p<0.008$). The goal level of non-HDL-C among patients with high cardiovascular risk (<2.6 mmol/l) during therapy was not achieved in anyone, and high risk (<3.4 mmol/l) was achieved only in 2 of 5 patients. The data obtained show that, despite the favorable changes in the lipid profile, many patients with FD on current therapy remain untreated; therefore, to increase the effectiveness of therapy, it is necessary to increase the dose of statin (in the absence of contraindications) and/or combine statins with fibrates.

Keywords: *apolipoprotein E, familial dysbetalipoproteinaemia, type III hyperlipoproteinaemia*

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INTRODUCTION

Familial dysbetalipoproteinemia (FD) or type III hyperlipoproteinemia (HLP) is remnant hyperlipidemia associated with genetic dysfunctional apolipoprotein (apo) E variants or its absence. ApoE is a key apoprotein for receptors (LDLR, HSPG-receptor, LRP) involved in elimination of very low density lipoproteins (VLDL) and chylomicrons (CM) remnants, i.e. residues of lipoproteins (LP) with high content of triglycerides (TG) formed by hydrolytical effect of lipoprotein lipase. Three basic isoforms of this apoprotein (apoE2, apoE3 and apoE4) are coded by three respective alleles (ε2, ε3 and ε4) of APOE gene. ApoE isoforms differ in 112 and 158 sites of amino acid sequence; so, apoE2 contains cysteine/cysteine, apoE3 – cysteine/arginine and apoE4 – arginine/arginine, respectively. ApoE3 is the most common isoform in the population; homozygosity by apoE2 is observed in more than 90% of patients with FD while apoE4 is associated with hypercholesterolemia and Alzheimer's disease [1]. Other genetic apoE variants or its complete absence are the cause of disease in approximately 10% of the patients with FD. Although ε2 homozygosity is observed in approximately 1% of persons in the population FD takes place only in 5% of ε2/ε2 carriers because the contribution of other genetic and environmental factors is required for full expression of the metabolic defect. Unrecognized FD is associated with high risk of both coronary and peripheral atherosclerosis because of accumulation of highly atherogenic remnant particles in the plasma. When FD is suspected diagnosis is easily confirmed by DNA analysis. Nevertheless, diagnosis of FD is rare because of insufficient awareness of physicians about this disease, low prevalence of the lipid metabolism disorder in the population and its masking by concomitant metabolic defects such as obesity, diabetes mellitus, hypothyroidism, etc.

MATERIAL AND METHODS

Patients

The study included 367 patients (155 males, mean age: 43.7 y, and 212 females, mean age: 52.5 y) who had available of DNA analysis. The group consisted of subjects with different HLP types who took medical advice for the first time or who had been already followed-up at the Myasnikov Institute of Clinical Cardiology. FD was diagnosed basing on the combination of clinical criteria (presence of mixed HLP) and genetic testing (presence of ε2/ε2 APOE genotype). The risk factors of FD included BMI >25 kg/m2, alcohol abuse (daily use of >20 and 30 g of pure alcohol for females and males, respectively), glucose metabolism disorder (diabetes mellitus or impaired glucose tolerance), hypothyroidism (data in the medical history or hormone replacement therapy at present). Diagnosis of coronary heart disease (CHD) was made in subjects with medical history of myocardial infarction, significant coronary artery atherosclerosis by coronarography, endovascular coronary interventions and coronary artery bypass grafting surgery. Presence of atherosclerotic plaques constricting the vascular lumen by more than 50% basing on the findings of visualizing investigations was considered as pathology of the peripheral arteries. The control group for comparison of the lipid profile included the subjects with plasma total cholesterol (TC) level less than 6.2 mmol/l and TG level less than 2.3 mmol/l, without chronic diseases, which can influence the lipid profile or require hypolipidemic therapy/administration of the drugs capable of exerting effect of lipid metabolism.

Measurement of blood serum lipids

Analysis of serum fasting TC, TG and high density LP cholesterol (HDL-C) was performed by the Architect C-8000 Biochemical Analyzer (Abbott, USA) using photometric methods with reagents of the equipment manufacturer. TC and TG levels were determined by the unified enzymatic method, HDL-C and LDL-C was measured by the direct homogenic methods. Non-HDLC was calculated using formula: TC – HDL-C.

Genotyping of rs7412 variant of APOE gene

Analysis for rs7412 variant of APOE gene was performed by the method of real-time polymerase chain reaction (PCR) using adjacent samples and melting of reaction products after PCR [2, 3]. The commercial Metabolism Genetics test-system (DNA-Technology, Russia, cat. No.: R1-H908-N3/4) was used. PCR was performed by of the DTprime Detecting Amplifier (DNA-Technology, Russia, cat. No.: O-DTPRIME4M1). The following temperature amplification mode was used: 94°C for 10 s, 64°C for 30 s, 50 cycles. After completion of amplification reaction, we obtained melting curves by increasing the reaction mixture temperature from 25°C to 75°C with increment of 1°C measuring the fluorescence level at each increment. The genotyping procedure was performed in two directions.

Statistical analysis

Statistical analysis was performed using STATISTICA 10.0 statistical software. The results are presented as mean and standard deviation (Mean ± SD). When comparing groups by quantitative variables, we used parametric (Student test for two independent groups) and nonparametric (Mann-Whitney test for two independent groups, analysis of variance ANOVA according to Friedman for three dependent groups) methods. Fisher's exact test was used when comparing groups by qualitative signs. Differences were considered as statistically significant at $p<0.05$.

RESULTS

The DNA analysis of 367 patients with different types of lipid metabolism disorders revealed eighteen ε2 homozygotes what was 4.9% of the total subject number. The clinical and demographic characteristics of identified patients with FD are presented in Table 1.

Table shows that subjects of opposite sexes were represented equally; the patients' at the first visit to the clinic coincided with the age when HLP was revealed for the first time; one half of the patients had at least one risk factor of FD and one third of the patients had 2-3 factors. CHD was revealed in 27.8% of the patients. Physical clinical symptoms of the disease are presented in Figure 1.

When comparing the baseline lipid profile of the patients with FD and control group, we found significantly higher TC, TG, LDL-C and non-HDL- C levels while HDL-C levels were considerably lower (Table 2).

We assessed also effects of the hypolipidemic therapy in 11 patients with FD. Among these, 8 patients were referred to the high cardiovascular risk category because they suffered from CHD or peripheral atherosclerosis, diabetes mellitus type 2. When interim results were estimated, 1 patient received monotherapy with fibrate, 3 patients received monotherapy with Atorvastatin in the dose of 10-

40 mg/day, 5 patients received monotherapy with Rosuvastatin in the dose of 10-40 mg/day and 1 patient was treated with combined therapy with Rosuvastatin (10 mg/day) and Fenofibrate. When the last results were estimated, 1 patient received monotherapy with fibrate, 4 patients received monotherapy with Atorvastatin in the dose of 40 mg/day, 4 patients received monotherapy with Rosuvastatin in the dose of 5-40 mg/day and 2 patients were treated with combined therapy with Rosuvastatin (10 mg/day) and Fenofibrate. Figure 2 presents changes in the lipid profile during the hypolipidemic therapy; visits 1 and 2 occurred with different intervals and reflected interim biochemical analyses and those which were the closest to the time of preparing the paper. Mean TC, HDL-C levels during the treatment decreased approximately 2 times as compared to the baseline (p<0.002), LDL-C level lowered 1.5 times (p<0.008) while the increase of HDL-C levels was statistically insignificant.

Table 1. Baseline clinical and demographic characteristics of identified patients with FD

Characteristic Value	Значение
Sex (m/f) 9/9	9/9
Age (years) 42.4±8.2	42,4±8,2
BMI 28.1±2.9	28,1±2,9
SBP (mm Hg) 134.1±14	134,1±14
DBP (mm Hg) 83.7±7.5	83,7±7,5
Alcohol abuse (%) 20	20
Smoking (%) 27.8	27,8
Family history of early CVD (%) 38.9	38,9
Age when FD was revealed (years) 42.4±8.2	42,4±8,2
Menopause in females (%) 55.5±1.5	55,5±1,5
Glucose metabolism disorder (%) 27.7	27,7
Hypothyroidism (%) 16.7	16,7
Number of patients having risk factors of FD (%): - 0 - 1 - 2 - 3	 11,1 55,5 27,8 5,5
Medical history of pancreatitis (%)	11,1
Peripheral atherosclerosis (%)	11,1
Clinical symptoms of FD (%): - tendon xan-thomas - corneal lipoid arch - skin xan-thomas - xanthelasmas - palm striae	 22,2 11,1 16,7 16,7 16,7
CHD (%)	27,8
C-reactive protein, mg/dl	0,24±0,19
Lipoprotein (a), mg/dl	48,8±64,7

The data is presented as mean ± standard deviation.
DBP = diastolic blood pressure, CHD = coronary heart disease, BMI = body mass index, SBP = systolic blood pressure; FD = familial dysbetalipoproteinemia; CVD = cardiovascular diseases

Table 2. Baseline (without treatment) lipid profile in the patients with FD

Parameter	Patients with FD (n=18)	Control group (n=43)	p-level
Age	42,4±8,2	43,5±14,2	н.д.
BMI	28,1±2,9	26,9±4,3	н.д.
TC	10,4±2,4	5,2±0,7	<0,00001
Triglycerides	5,96±2,6	1,19±0,5	<0,00001
HDL-C	1,02±0,2	1,38±0,3	<0,00001
LDL-C	4,5±1,3	3,2±0,8	<0,005
non-HDL-C	9,5±2,6	3,8±0,8	<0,00001

The data are present as mean ± standard deviation.

HDL = high density lipoproteins; LDL = low density lipoproteins; TC = total cholesterol

DISCUSSION

FD is a rare autosomal-recessive lipid metabolism disorder with variable penetrance with rate in the general population of about 1:1000 [4] but prevalence of this disease may be higher in some population groups, e.g., among subjects with mixed hyperlipidemia (i.e. with hypertriglyceridemia (HTG)) or in the patients with CHD/peripheral atherosclerosis. The rate of FD among the patients with pronounced HLP included in our study was 4.9% what is comparable with the data obtained in other populations with high cardiovascular risk. So, the prevalence of FD was 3.4% among 653 patients with familial forms of early CHD as compared to 1% in control group in a case-control study [5].

Some metabolic and genetic factors, more precisely, their interaction, predispose to FD development. In our study, patients' BMI was on average 28.1±2.9, glucose metabolism disorder was observed in 27.7% of the patients, hypothyroidism was found in 16.7% what is similar to the data obtained in a one-step crossover study including 305 patients with FD from four European populations: 28.5, 28.5% and 12%, respectively [4]. It is worth mentioning that we did not reveal evident glucose metabolism disorders, hypothyroidism, obesity or alcohol abuse in 40% of the patients; a considerable influence of other genetic factors, in particular mutations or genetic lipolysis system variants including APOC3, LPL, etc., on the development of FD is more probable in such cases [6].

Palmar xanthomas (called also flat palmar xanthomas or xanthoma striata palmaris) which are nodular lipid depositions of yellow color in the palm folds are practically pathognomonic for FD; they may look like only yellow coloring of the folds without nodules in some patients. Tuberous xanthomas (solid or soft nodules) or tuberous-eruptive xanthomas (eruptive xanthoma clusters resulting in enlargement of lesions elevated above the skin surface) are often revealed in untreated patients. They are located as a rule above the tuberosity of tibia, on the elbows and buttocks. One of the studies performed by American researchers reports the rate of palmar xanthomas of 64%, rate of tendon xanthomas – 23%, rate of tuberous or tuberous-eruptive xanthomas – 51% and the rate of eruptive xanthomas of 4% in the patients with FD [7]. In our study, palmar, tendon and tuberous-eruptive xanthomas were observed considerably rarer (16.7%, 22.2% and 16.7%, respectively) what may be explained by significant difference in the range of effective hypolipidemic drugs at present and previously when the foreign study was performed.

Atherosclerotic vascular lesions are the most important clinical manifestation and consequence of FD because VLDL and CM remnants with high cholesterol content are highly atherogenic particles. When the patients visited our clinic for the first time, hemodynamically significant pathology of the coronary or peripheral arteries was observed in one third of cases what indicates early development of the atherosclerotic cardiovascular disease taking into account the patients' mean age of 42.5 years. The data on the rate of CHD and peripheral artery involvement (27.8% and 11.1%, respectively) in the patients with FD included in our study are similar to the results obtained in the study performed by Koopal et

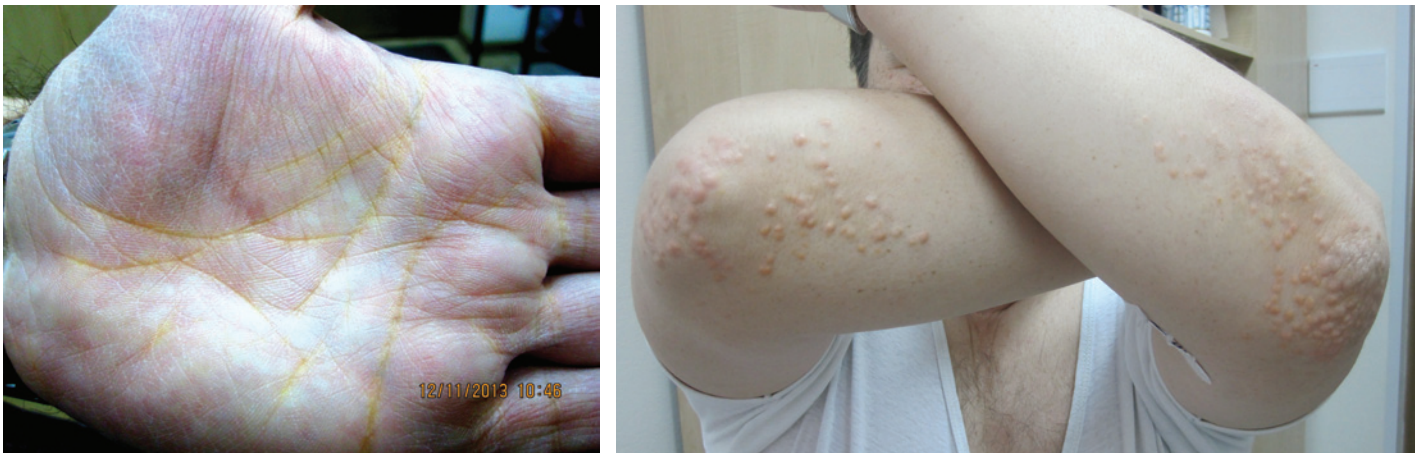

Figure 1. External clinical symptoms of type III HLP: palm striae (upper photo) and eruptive xanthomas on the elbows (lower photo)

al., where CHD and peripheral atherosclerosis were revealed with the rate of 19% and 11%, respectively [4].

Plasma non-HDL-C level reflects cholesterol content in all LP particles with high TG content including VLDL, CM and their remnants in addition to LDL. Modern practical guidelines recommend non-HDL-C as the better risk indicator than LDL-C [8]. This is especially urgent in some situations, in particular, when performing assessment in subjects with HTG when the use of Friedewald formula for estimation of LDL-C has certain limitations. On the other hand, assessment of cardiovascular risk in the patients with FD using only LDL-C will be incorrect because of the fact that ε2/ε2 genotype is usually associated with lower LDL-C levels as compared to other APOE genotypes [9]. The non-HDL-C level in the patients with FD was 2.5 times as high as that in control group in our study.

When considering pharmacotherapy of FD, one should mention that most patients usually react well to statins. If HTG predominated in the lipid profile, fibrates may be drugs of choice; combination of both drugs is required in some cases [10]. When assessing the effects of hypolipidemic therapy, most our patients (8 of 11) were referred to very high risk group; thus, the target non-HDL Chol level in them was <2.6 mmol/l. The analysis performed in our study showed that the target non-HDL-C level was reached in none of these patients. The target non-HDL-C (<3.4 mmol/l) was reached only in 2 of 5 patients among the patients with high cardiovascular risk. Most experts consider values of less than 1.7 mmol/l as normal plasma TG level [10]. Such TG level was reached in 27% of the patients receiving the therapy in our study. The plasma HDL-C levels of higher than 1 mmol/l in males and higher than 1.2 mmol/l in females are considered optimum [10]. In our study such levels were reached in 45%of the patients receiving the hypolipidemic therapy as compared to baseline value of 23%.

Thus, the data of this study shows that, in spite of significant favorable shift in the lipid profile caused by the hypolipidemic therapy, many patients with FD receiving the current therapy, remain treated insufficiently if we make judgement basing on such integral parameter as non-HDL-C. It is necessary to increase the dose of statin (up to maximum permissible and safe for a specific patient) and/or combine statin therapy with fibrates in order to enhance the efficiency of the therapy is such cases.

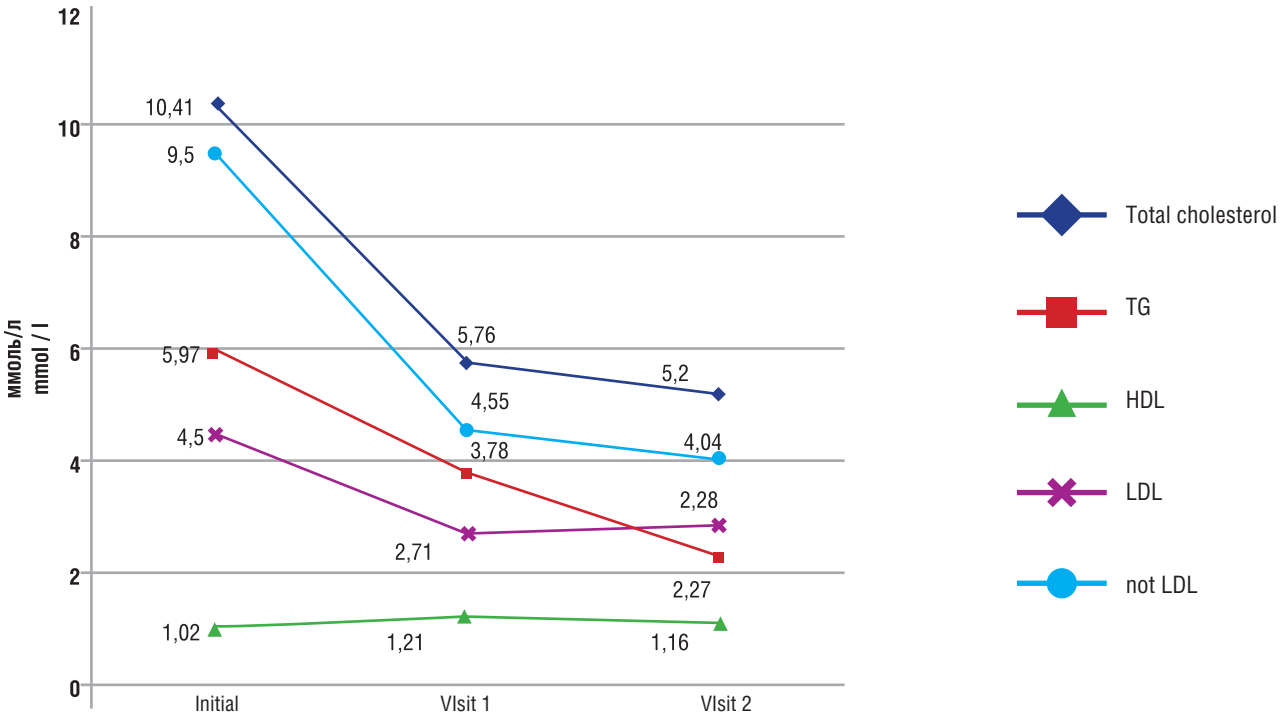

Figure 2. Changes of the lipid profile in the patients with type III HLP during the period of hypolipidemic therapy of different regimens (description see above)

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