

LPL gene mutation as the cause of severe hypertriglyceridemia in the course of ketoacidosis in a patient with newly diagnosed type 1 diabetes mellitus

Mutacja genu *LPL* jako przyczyna skrajnej hipertrójglicerydemii w przebiegu kwasicy ketonowej u pacjenta ze świeżo ujawnioną cukrzycą typu 1

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Abstract

Introduction. Severe hypertriglyceridemia is a condition associated with extremely high triglycerides (TG) plasma concentrations exceeding 1000mg/dl. This condition may result in mutations in genes encoding lipoprotein lipase (LPL), apolipoprotein C2 (APOC2) and apolipoprotein A5 (APOA5) characterized by an autosomal recessive inheritance pattern. **Aim.** A case report of a patient in which clinical picture of type 1 diabetes mellitus (T1DM) was accompanied by diabetic ketoacidosis (DKA) and severe hypertriglyceridemia. **Case report.** A 2.5-year-old boy was admitted to the hospital with ketoacidosis (pH – 7.0, BE – 20mmol/l, HCO₃ 10mmol/l), glucose level of 850mg%, hyponatremia (Na 100mmol/l) and hyperlipidemia (TG 13493 mg/dl, TC 734 mg/dl). The administered treatment resulted in nearly normal glycemic values and lipid disturbances normalization. This child was diagnosed with a heterozygous mutation of the *LPL* gene. Currently with an intensive insulin therapy and correct metabolic control of type 1 diabetes mellitus (T1DM), this patient maintains a normal lipid profile. **Conclusion.** In patient with T1DM the diagnosis of severe hypertriglyceridemia in the course of ketoacidosis should be based on careful interpretation of laboratory tests results. Moreover genetic tests of the patient and his/her immediate relatives blood samples should be performed.

Key words

hypertriglyceridemia, ketoacidosis, lipoprotein lipase, *LPL* gene mutation, APOC2

Streszczenie

Wstęp. Ciężka hipertrójglicerydemia to stan, w którym stężenie trójglicerydów (TG) przekracza 1000 mg/dl. Za zaburzenie to mogą odpowiadać mutacje w genie lipazy lipoproteinowej (LPL), apolipoproteiny C2 (APOC2) i apolipoproteiny A5 (APOA5) dziedziczone w sposób autosomalnie recesywny. **Cel.** W pracy przedstawiamy pacjenta, u którego manifestacji klinicznej cukrzycy towarzyszyła kwasica metaboliczna oraz skrajna hiperlipidemia. **Opis pacjenta.** 2,5 letni chłopiec przyjęty został do szpitala z kwasicą ketonową (pH – 7,0, BE -20 mmol/l, HCO₃ 10 mmol/l), stężeniem glukozy 850 mg%, hiponatremią (Na 100 mmol/l) oraz hiperlipidemią (trójglicerydy (TG) 13493 mg/dl, cholesterol całkowity (TC) 734 mg/dl). Zastosowano leczenie, uzyskując prawidłowe glikemie oraz normalizację zaburzeń lipidowych. U dziecka rozpoznano heterozygotyczną mutację genu *LPL*. Intensywna insulinoterapia i prawidłowe wyrównanie metaboliczne cukrzycy typu 1 gwarantują prawidłowy lipidogram u pacjenta do chwili obecnej. **Wniosek.** Rozpoznając skrajną hipertrójglicerydemie w kwasicy ketonowej w przebiegu cukrzycy typu 1, należy ostrożnie interpretować wyniki badań laboratoryjnych oraz dążyć do wykonania badania genetycznego u pacjenta, także u najbliższych jego krewnych.

Słowa kluczowe

hipertrójglicerydemia, kwasica ketonowa, lipaza lipoproteinowa, mutacja genu *LPL*, APOC2

Introduction

Diabetic ketoacidosis (DKA) is associated with an increase in triglycerides (TG), and total cholesterol levels (TC) and decrease in HDL cholesterol levels (HDL-C) [1]. The above mentioned TG increase is observed in approximately 30-50% of cases and results from temporary impairment of lipoprotein lipase (LPL) activity – a strictly insulin-dependent enzyme [1,2]. Cases of severe hypertriglyceridemia with TG levels exceeding 1000mg/dl are very rarely described (<1%), and most commonly result from *LPL* gene mutation, reduced LPL activity and deficiency of apolipoprotein C2 (APOC2) necessary for LPL activation [1]. Mutations responsible for increased serum TG levels were also observed in the gene encoding apoB-100 [3,4]. APOC2 is considered one of the most important physiological LPL activators [4]. Normal LPL functioning also requires the presence of other proteins inter alia: APOA5, GPIHBP1 (glycosylphosphatidylinositol-anchored-HDL-binding protein) and ANGPTL4 (angiopoietin protein-like 4) [4,5]. Chylomicrons and VLDL transfer APOC2 and APOA5 apolipoproteins that bind to GPIHBP1, forming a platform for LPL binding with its substrates. Indeed, serum LPL activity is the final effect of the interaction between LPL's activator – apolipoprotein C2 (APOC2) – and inhibitor – apolipoprotein C3 (APOC3). Furthermore, LPL expression is enhanced by insulin [5].

The aim of this article is to present a case report of a 2.5-year-old boy, in whom diabetic ketoacidosis accompanying type 1 diabetes mellitus diagnosis resulted in severe hyperlipidemia due to *LPL* gene mutation.

Case report

An on-term, naturally delivered child of young and healthy parents with a body mass of 3.350 g, body length 53-cm, and an Apgar score of 9 points. His psychomotor development was normal. During the medical interview no relevant diseases were noted. Approximately one month before diagnosis this 2.5-year-old boy presented following signs and symptoms: polydipsia, polyuria and rapidly progressing weight loss. With regard to health deterioration he was admitted to the district hospital, where T1DM accompanied by severe DKA was diagnosed (pH-7.0, BE -20mmol/l, HCO₃ 10-mmol/l, pO₂ 70-mmHg, pCO₂ 40-mmHg, glucose level 850mg%). Sodium levels (measured

several times) were as follows: 100, 99, 134-mmol/l. Levels of potassium, TG and TC were – 4.4-mmol/l, 13493-mg/dl and 734-mg/dl respectively.

Taking into account the child's serious health condition and his age, this patient was transferred to the pediatric intensive care unit, where insulin therapy and intravenous fluid therapy were initiated. The initiation of parenteral nutrition resulted in nearly normal glycemic values and metabolic improvement. TG and TC levels were progressively decreasing to: 7019mg/dl, 4025mg/dl (TG) and to 615 mg/dl (TC). Partial plasmapheresis resulted in marked decrease of TG and TC levels to: 1172mg/dl, 690mg/dl (TG) and 272mg/dl, 192mg/dl (TC).

Patient's status on admission to the clinical unit was as follows: body weight 11 kg (10-25pc), pH and BE normal, HbA1c 11.6%, TC 367mg/dl, TG766 mg/dl, HDL-C-24 mg/dl. Abdominal ultrasound revealed hepatic enlargement and increased echogenicity points towards hepatic steatosis. An intensive insulin therapy resulted in improvement of the child's condition. Therefore on the second day of hospitalization oral food intake was initiated. With regard to persistent hypertriglyceridemia (493mg/dl), a treatment with atorvastatin (2.5mg/dobę) was started. It was withdrawn after 2 weeks, resulting in blood TG levels normalization. In the patient's diet, animal fat intake was reduced with simultaneous increase in omega-3 fatty acid intake. Six months of treatment resulted in normalization of lab test results and liver ultrasound image and this state remains unchanged until this day. Table I summarizes the patient's results.

Molecular tests using RFLP (Restriction Fragment Length Polymorphism) and ASA-PCR (Allele Specific Amplification Polymerase Chain Reaction) methods of this boy's and his parents' blood samples did not confirm the presence of a common gene mutation in *ApoB100* or the presence of G571E mutation in the *LDRL* gene. Moreover, molecular tests using sequencing techniques directly towards mutations did not detect any mutation in lipoprotein lipase (*LPL*), apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*) genes as well as in apolipoprotein E (*APOE*) gene exon 4. Nevertheless, molecular analysis using MLPA method to detect microrearrangements (Multiplex Ligation-dependent Probe Amplification) revealed a heterozygous mutation in *LPL* gene. Results of this patient's genetic tests are summarized in Table II.

The patient's father also presented with some lipid profile abnormalities (TC 230mg/dl, TG 189mg/dl), however in this

Table I. Cholesterol and triglycerides levels in patient during following days of treatment

Tabela I. Stężenie cholesterolu i triglicerydów u pacjenta w kolejnych dniach leczenia

	25.04. 2011	25.04 2011 10 p.m.	26.04 2011	26.04 2011 1 p.m.	26.04 2011 10 p.m.	27.04 2011	28.04 2011	13.05 2011	01.09 2011	19.12 2011
TC	734	-	615	-	274	194	337	375	180	154
TG	13493	7019	-	4025	1172	690	766	495	90	178

Table II. Results of molecular analysis in presented patient
Tabela II. Wynik analizy molekularnej u przedstawianego pacjenta

Analysed gene	Scope of analyse	Test method	Mutation (bold letter) and polymorphisms
<i>ApoB100</i>	Mutations: R3500Q, R3531C, H3543Y	RFLP	-
<i>LDLR</i>	Mutation: G571E	ASA-PCR	-
<i>LPL</i>	Mutations in exon 6	Direct sequencing	
<i>APOC2</i>	Mutations in exons 1 and 2	Direct sequencing	Rs2288912 C>G Rs10422603 T>G Rs2288911 T>G
<i>APOA5</i>	Mutations in exons 1 and 2	Direct sequencing	Rs662799 C>T* Rs651821 G>A RS 3135506 C>G*
<i>LPL</i>	Microrearrangements in <i>LPL</i> gene – microdeletion and microduplication	MLPA	c.1018+34 A>G heterozygote

* Polymorphic variant potentially impacting high blood serum TG level occurrence in studied patient.

case no *LPL* gene mutation was confirmed. In contrast, genetic tests of the mother's blood sample revealed the presence of above mutation. Nevertheless, her TC and TG levels have always been normal.

Discussion

Severe hypertriglyceridemia is most commonly accompanied by acute pancreatitis and/or hyponatremia [6,7]. In children with DKA, those disturbances are present in 4-7% of cases, but compared to adults are rarely diagnosed [7-9]. Authors emphasize that hypertriglyceridemia (TG 200-1000mg/dl), present in the initial phases of acute pancreatitis (AP), is not a direct reason for AP development, but rather a consequence of pancreatic dysfunction [7]. In many cases of hypertriglyceridemia with or without concurrent DKA, AP was not present [1,5,10-13]. In our patient, AP was also not detected. According to literature data, TG levels of > 360mg/dl render an execution of reliable blood electrolytes measurements impossible. This situation was observed in our patient as well as in the case of a patient presented by Turkish authors [14]. In the available literature, a pseudohyponatremia resulting from the presence of high lipid levels in dense milky serum is most commonly reported. Moreover, falsely low readings of haemoglobin and bilirubin are also observed [14,15]. Furthermore, a concurrent pseudonormoglycemia may also delay diagnosis and treatment of a pediatric patient with DKA [1,16]. Many researchers believe that severe hypertriglyceridemia results from genetic disturbances leading to reduction in *LPL* activity or deficiency of it [17]. In our patient, *LPL* gene mutation

was detected, enabling type IA hypolipoproteinemia. In general, the above mentioned mutation type is associated with an excessive chylomicron level in dense and milky blood serum [17]. In the available literature, over 100 mutations of that gene with autosomal recessive inheritance pattern were described [3,17]. In a given population, the frequency of individual *LPL* gene mutations varies. Ser477stop and Asp9Asn mutations are the most common. In the Caucasian population, those mutations represent 2 to 5% of all heterozygotes. The majority of the above mentioned mutations are responsible for different degrees of decrease in *LPL* enzymatic activity [13], whereas Ser477stop polymorphism is considered a protective mutation and prevents the development of lipid disturbances [3,13]. The protective effect of this polymorphism can be modified and attenuated by environmental factors, concomitant carbohydrate metabolism disorders and metabolic syndrome [13]. However, in the presented case, the above mentioned polymorphisms were not analysed.

In the early childhood, patients with homozygous mutation of the *LPL* gene present with severe hypertriglyceridemia accompanied by abdominal pain, hepato- and/or splenomegaly, recurrent AP episodes and cutaneous lipomas [11,12]. In approximately 25% of patients, those symptoms occur during the first year of life and in the remaining group they develop until the age of 10. Abdominal pain is the most commonly observed [15]. The estimated population frequency of heterozygotes is 1 in 500 cases [11].

In heterozygotes with normal health condition, blood lipid level is normal or slightly increased, like in our patient and his mother. In those patients, disease symptoms may occur during periods of insulin deficiency, similar to the case of presented

patient. The symptoms may also manifest during stressful situation or pregnancy [17,18]. Heterozygotes may also exhibit other *LPL* gene mutations resulting in extremely increased serum lipid levels observed during DKA associated with newly-diagnosed T1DM and in chronic DM with poor metabolic compensation [17]. Heterozygotes, regardless of the presence of clinical symptoms, have a higher risk of developing cardiovascular (CV) diseases than the healthy population. Off note, children with low birth weight have a higher risk of developing metabolic syndrome than children with normal birth weight [3,18].

In the presented case, no *LPL* gene mutation-associated symptoms were observed before the occurrence of DM. Therefore, before DM diagnosis, no lipid profile measurements were performed, though they would probably be normal. This is demonstrated by the fact that normalization of DM metabolic control resulted in the maintenance of normal TC and TG levels. The use of lipid-lowering agents, such as statins or fibrates, in patients with lipoprotein lipase deficiency is not as beneficial as in subjects with other conditions accompanied by hyperlipidemia. [18,19]. Therefore, the improvement of our patient's lipid profile after 2 weeks of therapy with statins probably depended more

on appropriate insulin therapy and balanced nutrition. Blackett *et al.* confirmed that intensive insulin therapy ensures normolipidemia without the necessity of a low-fat diet [1].

In general, plasmapheresis is not the standard treatment. However, in severe hypertriglyceridemia, especially in the course of AP episodes, it may be useful for rapid blood TG level reduction, if initiated within 24 hours after diagnosis [20]. Plasmapheresis as well as statin treatment should not be considered a routine management of patients with familial *LPL* deficiency at any stage of this disorder [20].

Conclusion

In DKA patients with severe hypertriglyceridemia occurring in the course of T1DM, it is important to carefully interpret laboratory test results, and to perform appropriate genetic testing. Moreover, genetic counselling should also be offered to first-degree relatives.

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