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Case report

Prenatal diagnosis of Smith-Lemli-Opitz syndrome based on recognition of fetal ambiguous genitalia in association with congenital heart disease

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Abstract

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disease caused by a mutation in the 7-dehydrocholesterol reductase gene (*DHCR7*) on chromosome 11, which leads to 7-dehydrocholesterol reductase enzyme defects. This results in the building up of toxic by-products of cholesterol production in the blood and the nervous system. All this affects the growth and development of human body systems. We presented a case of a fetus of a pregnant woman with a high-risk pregnancy after IVF. The patient was referred for a fetal karyotype test, which showed a male sex (46, XY). This woman was referred to our centre for fetal heart evaluation. Based on our protocol established at the Department of Prenatal Cardiology of the Institute of the Polish Mother's Health Centre in Lodz, our examination starts with obstetrical screening, including ultrasound fetal gender evaluation. At this point of examination, the fetus presented ambiguous genitalia and fetal polydactyly. The fetal echocardiographic examination revealed atrioventricular septal defect. The presence of ambiguous genitalia and atrioventricular septal defect allowed us to suspect SLOS. As a result, further diagnostic steps were suggested, and the diagnosis was confirmed. The family chose comfort care with no postnatal intervention and planned the next pregnancy with another donor. We would like to underline the importance of a detailed ultrasound protocol before fetal echocardiographic examination for its proper interpretation.

Key words: SLOS, prenatal cardiology, genetics, prenatal diagnosis, heart defects, fetal genitalia, ambiguous genitalia.

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Introduction

Mutations in the 7-dehydrocholesterol reductase gene (*DHCR7*) on chromosome 11 result in cholesterol deficiency, which leads to developmental disorders of a fetus and a number of abnormalities constituting the components of Smith-Lemli-Opitz syndrome (SLOS). The cholesterol deficiency results from the lack of 7-dehydrocholesterol reductase, which leads to a build-up of toxic by-products of cholesterol production. The by-products accumulate mainly in the blood and

the nervous system. All this affects the growth and development of many body systems. Children with SLOS who present very low levels of cholesterol may die due to internal issues. However, proper and early diagnosis and treatment make it possible for children to live for a long period of time and have a normal adulthood [1]. One of the signs of SLOS may be a discrepancy between fetal sex evaluated by genetic examination and by ultrasound examination [2, 3]. The ultrasound examination of the fetal genitalia before a detailed fetal heart evaluation may be helpful in interpreting the fetal echocardiography examination in a proper manner. SLOS may be accompanied by congenital heart defects along with functional abnormalities during fetal life. One of them may be atrioventricular septal defect; therefore, a detailed ultrasound of the fetus organs before fetal echocardiographic examination makes it possible to achieve a proper diagnosis.

Material and methods

This was a single-centre analysis of a single case of a fetus that had an ultrasound echocardiographic examination in our tertiary centre in Lodz in March 2020. The echocardiographic examination was performed by an experienced prenatal echocardiographer with the use of the Voluson E10 ultrasound machine. The fetus was in a cephalic presentation. The method used for steroid detection in the maternal urine sample was gas chromatographic/mass spectrometric (GS/MS) analysis. The following indices of the steroid metabolites were used for analysis of the maternal urine sample: 7-dehydropregnantriol [μg/g]/pregnantriol [μg/g], 8-dehydropregnantriol [μg/g]/pregnantriol [μg/g], 8-dehydroestriol [μg/g]/estriol [μg/g].

Case presentation

This case involved a pregnancy after obtained by an in vitro fertilisation (IVF) in a 35-year-old female multigravida with a medical report of 4 miscarriages in the first trimester and one birth of a healthy child after previous IVF procedures. The patient had one examination performed in our centre at 32 weeks of gestation based on ultrasound biometry (39.3 weeks of gestation based on the last menstrual period). In the first trimester of the considered pregnancy, an increased risk of trisomy 13 (1:24) was considered based on the first trimester screening program of the Fetal Medicine Foundation software for risk calculation, which included maternal age, fetal nuchal translucency, nasal bone, crown-rump length, and fetal heart rate. Bearing in mind the above-mentioned findings, amniocentesis at the 15th week of gestation was performed. The amniocentesis showed normal male karyotype results (46, XY) based on the quantitative fluorescent polymerase chain reaction. The ambiguous genitalia had not been previously recognised due to oligohydramnios and an unfavourable position of the fetus in the uterus.

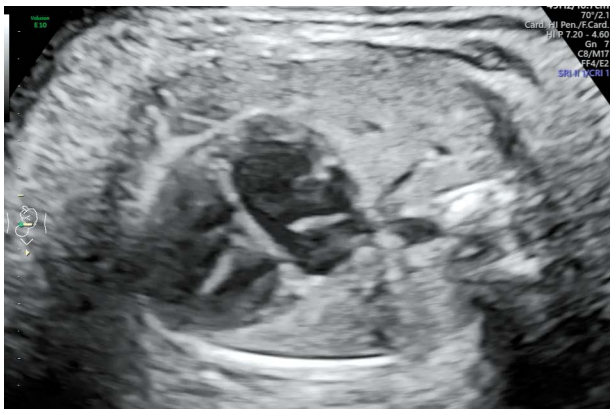

Figure 1. Atrioventricular septal defect at the 32nd week of gestation in 2D presentation

The amniotic sample was analysed only for trisomy risk assessment. At the 25th week of gestation, an abnormal fetal heart presentation was observed, and a detailed fetal echocardiographic examination was proposed. According to our protocol established at the Department of Prenatal Cardiology of the Institute of the Polish Mother's Health Centre in Lodz, we started our examination with obstetrical screening, including ultrasound fetal gender evaluation. At this point, fetal genitalia ambiguity was found. The placenta was located on the anterior wall of the uterus. The pulsatility index (PI) of the umbilical artery was 0.94. The PI of the middle cerebral artery was 2.63 with a maximum blood flow velocity of 33 cm/s. The heart-to-chest area ratio (HA/CA) was 0.4, while the heart transverse diameter was 40 mm.

Detailed fetal echocardiography revealed complete atrioventricular septal defect (AVSD) (Figure 1). However, in addition to structural problems of the heart, there were also functional abnormalities, including mild cardiomegaly, pericardial effusion, and mild common atrioventricular valve regurgitation. Therefore, the Cardiovascular Profile Score (CVPS) was calculated, at 6/10 (1 point for cardiothoracic area ratio, 1 point for pericardial effusion, 2 points for mild common atrioventricular valve regurgitation). During echocardiographic examination the function of the right ventricle was evaluated, and the RV Tei index was 0.73.

With the overall image of our case and consideration of other possible syndromes, all the information was gathered and analysed. All the information was presented and explained to the patient.

The pregnant woman was informed about the observation of a female fetus during an ultrasound examination. After double checking the earlier karyotype report, due to fetal gender discrepancy, it was decided to perform a maternal urine test to detect fetal steroid 7,8-dehydrometabolites. The 7,8-dehydrometabolites are considered to be markers of SLOS.

The urine test results presented elevated values of fetal steroid 7,8-dehydrometabolites:

7-dehydropregnantriol [μg/g]/pregnantriol [μg/g] = 0.4830 (reference values: 0-0.0140),

8-dehydropregnantriol [μg/g] / pregnantriol [μg/g] = 1.4632 (reference values: 0-0.0140), and

8-dehydroestriol [μg/g]/estriol [μg/g] = 0.34606 (reference values: 0-0.0190).

The presence of fetal genitalia ambiguity (Figure 2) and atrioventricular septal defect with functional abnormalities

(mild cardiomegaly, pericardial effusion, and common atrioventricular valve regurgitation), as well as the urine metabolite test results, suggested SLOS. Comfort care with no postnatal intervention was discussed and accepted by the pregnant woman. She did not opt for termination of pregnancy at that stage.

Table 1 presents all possible abnormalities that can be found in the course of SLOS. The abnormalities presented in our case are underlined. At the 32nd week of gestation, fetal growth restriction was observed. What is more, right foot polydactyly and second and third toe syndactyly were present. Other abnormalities included shortening of upper and lower extremities, parietally narrowed facial shape, cleft of the upper lip, female gender, right-sided adrenal hypertrophy (10 mm), right renal pyelectasis (14 mm), significant dilatation of the large intestine (21 mm), calcifications in the liver, dilated stomach with hyperechoic content, spherical and wide placenta (front wall wide placenta, spherical, additional lobe on the right side, numerous calcifications and vacuoles), and peripheral umbilical cord attachment to the placenta.

In this case, we observed additional cardiac abnormalities unrelated to an isolated heart defect, including heart enlargement with hypertrophy of the right ventricle myocardium, disproportion at the level of a four-chamber, and 3-vessel views with the dominance of the right side of the heart, as well as pericardial effusion. Colour Doppler flows showed mild common atrioventricular valve regurgitation (ASD + VSD = 7 mm). At the level of the atria, we observed an abnormal and accelerated left-right reversal flow with the predominance of the right atrial contractility. A postnatal echocardiogram was not performed because the family chose non-intervention/comfort care only.

The child was born at the 40th week of gestation by caesarean section due to a 1.6-mm scar after a previous caesarean section. The birth weight of the neonate was 3000 g, and the neonatal Apgar score was 5/5/6/6. The umbilical cord blood pH was 7.32, whereas alkaline deficiency was at the level of –0.5. The newborn presented dysmorphic features, fleshy ears, cleft palate, shortened bones of long limbs, hand and foot polydactyly, and adhesions of II-III and IV-V toes. Comfort care with no postnatal intervention was provided as decided before. The newborn was pronounced dead on the ninth day of postnatal life. Autopsy was declined by the parents. A genetic test showed that the deceased newborn had a pathogenic variant of c.452G>A (p.Trp151Ter) in both alleles of the *DHCR7* gene.

Discussion

We presented a case of a fetus of a pregnant woman with a high risk pregnancy after IVF, who was referred to our centre for fetal heart evaluation. Based on our protocol established at the Department of Prenatal Cardiology of the Institute of the Polish Mother's Health Centre in Lodz, we started our examination with the obstetrical part, which included ultrasound fetal gender evaluation, and, at this point, an abnormality in the form of ambiguous genitalia between karyotypic gender and gender based on ultrasound examination of the genitalia was found. During ultrasound examina-

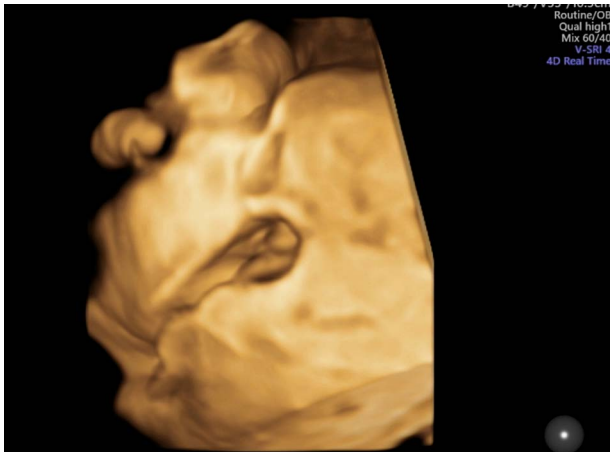

Figure 2. Fetal female-like genitalia in a male fetus according to the karyotype at the 32nd week of gestation

Table 1. A list of abnormalities present in SLOS that can be diagnosed in the prenatal period based on echo-sonographic examinations [1]. The most common ones are underlined

Fetal anatomy	Abnormalities with details
Head/neck	• Microcephaly (small head) • Bitemporal narrowing (reduced distance between temples) • Brain malformations including agenesis of the corpus callosum and, in very severe cases, major malformation of the front part of the brain (holoprosencephaly) • Cerebellar hypoplasia
Face	• Ptosis (drooping eyelids) • Epicanthal folds (skin folds of the upper eyelid) • Short and upturned nose • High-arched, narrow, hard palate • Cleft palate • Cataracts • Low-set and posteriorly rotated ears • Micrognathia (small chin) • Eye abnormalities
Thorax/heart	• Congenital heart defect • Pulmonary abnormalities
Abdomen	• Renal, adrenal, liver abnormalities • Hypospadias (genital malformation in boys) • Undescended testicles in boys • Pyloric stenosis • Bowel atresia • Hirschsprung's disease
Gender	• Ambiguous or female-like male genitalia
Extremities	• Syndactyly of the second and third toes (fused toes) • Polydactyly of hands or feet • Short, proximally placed thumb • Abnormal palmar creases (usually single)
Placenta, umbilical cord	• Spherical, wide placenta • Peripheral cord attachment to the placenta

tion, fetal polydactyly was also noticed. Due to the presence of such abnormalities, detailed fetal echocardiography was performed, which revealed an atrioventricular septal defect. Up to 45% of prenatally diagnosed cases with AVSD may have Down syndrome or left atrial isomerism (which usually coexists with normal karyotype). However, an isolated AVSD during fetal and neonatal life is usually clinically silent, and cardiac surgery is not required during the first month of postnatal life. Due to the presence of ambiguous genitalia, which was observed during obstetrical ultrasound examination, as well as atrioventricular septal defect in echocardiographic examination, some disorders that include ambiguous genitalia and AVSD were taken into consideration, including SLOS, CHARGE syndrome, and Kabuki syndrome. This is important because in most cases congenital heart defects constitute isolated malformations; however, it is reported that around 33% of all congenital heart defects are accompanied by other anomalies. Kabuki syndrome is characterised by facial abnormalities, mental retardation with a delay in postanal growth, as well as skeletal malformations and abnormalities [4]. It was important to find out which conditions and syndromes may have the same or similar presentation as found in our case. The presence of genitalia ambiguity and atrioventricular septal defect may be found in SLOS, CHARGE syndrome, Kabuki syndrome, Meckel syndrome, Pallister Hall syndrome, pseudo-trisomy 13 syndrome, squalene synthase deficiency, which is important for differentiation [5-12].

The spectrum of abnormalities observed in the fetus was wide, but the first abnormality that prompted us to perform further genetic diagnosis was fetal ambiguous genitalia due to extreme under-virilisation of the external genitalia before taking a detailed fetal echocardiographic examination. Pursuant to current recommendations, prenatal ultrasound diagnostics of external genitalia in the fetus is not obligatory and is usually recommended only in medically indicated situations, including multiple pregnancies [13]. According to our protocol, our examination always starts with obstetrical screening, including fetal gender evaluation.

In general, a preliminary gender analysis is possible in the first trimester based on observations of orientation of the genital tubercle in the mid-sagittal plane [14]. Measurement of the distance between the posterior wall of the urinary bladder and the anterior wall of the rectum in the fetus allows differentiation between the sexes due to the presence of the uterus in female fetuses, which lengthens the mentioned distance. According to the study by Chitayat et al., in male fetus, the distance between the posterior serosal surface of the bladder and anterior serosal surface of the rectum is smaller than in the female fetus. In the female fetus, the presence of the uterus leads to a convex indentation within the posterior aspect of the bladder wall [15]. Detailed reports related to descriptions of the gender of genital organs are not used in clinical practice [16, 17]. Based on retrospective analysis of medical records related to cases of chromosome-phenotype incompatibility detected prenatally, Adam et al. concluded that the diagnosis of the development of the sex disorders was possible in 24% of cases, while the most common postnatal diagnoses included penoscrotal hypospadias with transposition of the scrotum with unknown genetic cause and 21-hydroxylase deficiency [18].

In our case, the deceased child had a homozygous nonsense variant of *DHCR7*:c.452G>A (p.Trp151Ter). The aforementioned result confirmed the diagnosis of SLOS. The metabolic basis for SLOS is an abnormal cholesterol synthesis. The SLOS may be accompanied also by aortic coarctation, hypertelorism, and anteverted nares [19, 20]. Cholesterol is an essential component of the cell membrane and tissues of the brain. If a fetus synthesises low levels of cholesterol, it usually presents poor growth and postnatal developmental delays as well as mental retardation. It must be noted that steroid profiling in maternal urine is a reliable method of prenatal diagnosis for SLOS and is a statistically important test in this syndrome [21]. SLOS may also be detected using the biochemical GC/MS sterol analyses of amniotic fluid [22, 23].

It is important to remember that SLOS can be accompanied by many cardiac defects such as atrioventricular canal, primum atrial septal defect, patent ductus arteriosus at term, and membranous ventricular septal defect. All of them can be observed during prenatal echocardiographic examinations. In addition to structural heart defects, the literature describes cases of pulmonary hypertension in the newborn, which affects the postnatal life and becomes persistent hypertension. Most SLOS patients present renal anomalies, including renal hypoplasia or aplasia, renal cortical cysts, hydronephrosis, renal ectopia, ureteral duplication, and persistent fetal lobation [24].

What is important in our case is the evaluation of the fetal genitalia before performing a fetal echocardiographic examination. It can be said that congenital heart defects accompanied by genetic disorders such as SLOS and heart structural problems along with functional abnormalities during fetal life make it possible to predict a poor short-term prognosis, despite the fact that an isolated structural heart defect (AVSD) is not a life-endangering condition. The fetal heart issue was diagnosed as the first one and is additionally highlighted. According to the guidelines, the addition of the information about the gender in an ultrasound report is not obligatory. However, in the presented case, it was crucial information to suspect SLOS, which allowed us to decide to perform maternal urine analysis.

It is worth adding that there are reports on the possibility of intrafetal cholesterol supplementation, which, of course, cannot cure the enzymatic defect but can alleviate some congenital effects through fetal intravenous and intraperitoneal transfusions of fresh frozen plasma, which increases the level of fetal cholesterol measured in cordocentesis samples [25]. What is more, cholesterol supplementation improves fetal red cell volume, which provides better oxygenation of the tissues. However, dietary cholesterol does not cross the blood brain barrier. This may suggest that therapeutic intervention in SLOS is possible, but further research is required [25].

Conclusions

To conclude, we would like to underline the importance of a detailed ultrasound protocol before fetal echocardiographic examination for its proper interpretation. A detailed ultrasound examination of all the structures, including the genitalia before echocardiographic screening, is crucial because proper interpretation of the fetal echo can only be possible when other fetal structures are observed and analysed. When all the elements are combined, SLOS syndrome can be one of the suggested possible findings, which requires further genetic diagnosis. In the overall diagnosis, the importance of multidisciplinary collaboration among fetal cardiologists, obstetricians, maternal-fetal-medicine specialists, and geneticists/genetics counsellors must be emphasised.

Conflicts of Interest

The authors declare no conflict of interest.

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