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PRACE ORYGINALNE
położnictwo

Trends in prenatal diagnosis of non-specific multiple malformations disorders with reference to the own experience and research study on Smith-Lemli-Opitz syndrome

Trendy w diagnostyce prenatalnej niespecyficznych zespołów wad rozwojowych z uwzględnieniem doświadczeń własnych i badania nad częstością prenatalną zespołu Smitha, Lemlego i Opitza

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Abstract

Aim of the study: *Biochemical diagnosis of fetuses with multiple malformations – an attempt to determine the frequency of prenatal Smith-Lemli-Opitz syndrome. Discussion on trends in prenatal diagnosis of non-specific multiple malformations disorders.*

Material and methods: *A total of 117 fetal samples were obtained. They were analyzed with gas chromatography/mass spectrometry (GC/MS) method to assess the concentration of 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC) in amniotic fluid samples and(or) to establish 7-dehydroestriol/estriol and 8-dehydropregnanetriol/pregnanetriol ratios in maternal urine.*

Results: *In 4 cases Smith-Lemli-Opitz syndrome was confirmed.*

Conclusions: *Biochemical GC/MS sterol analyses of amniotic fluid or maternal urinary metabolites toward Smith-Lemli-Opitz syndrome, as cheap tests, should be performed in all pregnancies with suggestive ultrasound features (holoprosencephaly and(or) atrioventricular canal and(or) genital anomalies), especially when nuchal translucency is increased >3mm, and after exclusion of chromosomal aberration in routine karyotyping or even arrayCGH.*

Key words: **Smith-Lemli-Opitz syndrome / prenatal diagnosis / GC/MS / aCGH /**

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Streszczenie

Cel pracy: *Diagnostyka biochemiczna płodów z mnogimi wadami rozwojowymi – próba określenia prenatalnej częstości zespołu Smitha, Lemlego i Opitza. Omówienie trendów w diagnostyce prenatalnej niespecyficznych zespołów wad rozwojowych.*

Mmateriał i metody: *Przeprowadzono 117 diagnostyk prenatalnych, obejmujących badania biochemiczne metodą GC/MS (chromatografia gazowa sprzężona ze spektrometrią mas) celem określenia: stężenia 7- i 8-dehydrocholesteolu w płynie owodniownym oraz wskaźników 7-dehydroestriol/estriol i 8-dehydropregnantriol/ pregnantriol w próbce moczu kobiet ciężarnych.*

Wyniki: *Potwierdzono 4 przypadki zespołu Smitha, Lemlego i Opiza.*

Wnioski: *Diagnostyka biochemiczna metodą GC/MS celem oceny specyficznych steroli w płynie owodniowym lub metabolitów w moczu kobiety ciężarnej w kierunku zespołu Smitha, Lemlego i Opitza, jako badanie tanie, powinna być wykonana w każdym przypadku gdy na podstawie badania USG u płodów stwierdzono: holoprosencefalię i(lub) kanał przedsionkowo-komorowy i(lub) anomalie zewnętrznych narządów płciowych, zwłaszcza przy zwiększonej przezierności karkowej (>3 mm) i po wykluczeniu aberracji chromosomowych w klasycznym badaniu cytogenetycznym czy nawet metodą mikromacierzy (array CGH).*

Słowa kluczowe: **zespół Smitha / Lemlego i Opitza / diagnostyka prenatalna / / GC/MS / aCGH /**

Introduction

The development of modern molecular technique such as microarray-based comparative genomic hybridization (array-CGH) has revolutionized clinical cytogenetics, as it provides a relatively quick method to scan the genome for gains and losses of chromosomal material with significantly higher resolution than was previously possible [1]. Postnatal chromosomal array method offer much greater diagnostic yields (15-20%) than G-banded karyotyping does for genetic testing of individuals with unexplained developmental delay, intellectual disability, dysmorphic features, autism or multiple congenital anomalies (~3%, excluding Down syndrome and other recognizable chromosomal syndromes), and is recommended by the International Standards for Cytogenomic Array Consortium as the first-tier cytogenetic tool for patients with those disorders [2, 3, 4].

Several reports have now shown the potential utility of arrayCGH in prenatal diagnosis; especially discussed is whether microarrays should be an attractive option for any woman undergoing genetic amniocentesis/CVS or only for pregnancies with abnormal ultrasound findings [5, 6, 7, 8]. To date, the rate at which arrayCGH can detect clinically significant copy number alterations in fetuses with ultrasound anomalies ranges from 2% to 16%, depending on the types of these anomalies [9, 10, 11, 12, 13].

According to the recommendations of the American College of Obstetricians and Gynecologist, conventional karyotyping should remain the principal cytogenetic test in prenatal diagnosis, and arrayCGH should be an adjunct to prenatal care for woman with abnormal ultrasound findings and a normal conventional karyotype [14]. However, the use of microarray-based comparative genomic hybridization only when the ultrasound abnormalities are present may limit the diagnostic potential of this assay [9]. It is because of possible problems in interpretation of the results. It can happen due to limited knowledge on the natural history and range of clinical variability associated with some described or previously undetected submiscroscopic deletions and duplications. The clinical applicability of array CGH technique in prenatal diagnosis seems well established for refining diagnosis in cases with suspicious or inconclusive diagnosis of chromosomal structural change. However larger population-based studies, with samples processed for both arrayCGH and conventional cytogenetic analysis, are still necessary before arrayCGH can be recommended as a first-line test in routine prenatal diagnosis practice for identification and molecular characterization of chromosomal abnormalities in the fetuses [14].

Moreover, the presence of a aberration alone does not necessarily mean that the copy number alteration causes the observed phenotype and is not equivalent to consider a copy number imbalance as to be pathogenic on the basis only of the association with fetal malformation indentified by ultrasound examination.

In the diagnosis of specific fetal malformations' causes, except chromosomal aberrations, one should remember also about monogenic disorders which obviously are not possible to be detected with array CGH. In the pregnancies with normal karyotype (46,XX or 46,XY) complicated by: increased nuchal translucency and(or) holoprosencehaly and (or) atrioventricular septal defect and(or) sex differentiation anomalies Smith–Lemli–Opitz syndrome (SLOS) should be considered.

SLOS is an autosomal recessive, multiple congenital malformation/intellectual disability disorder, caused by deficiency of 7-dehydrocholesterol reductase (DHCR7) that results in an accumulation of 7- and 8-dehydrocholesterol (7-and 8-DHC) in body tissues and fluids. In urine of pregnant women carrying a fetus affected with SLOS such metabolites as 7,8-dehydroestriol and 7,8-dehydropregnanetriol were identified about 12 weeks of gestation. These steroids are produced in placental-maternal unit from 7- and 8-DHC synthesized by adrenals of SLOS affected fetuses. Identification of this steroids in maternal urine is used for early non-invasive prenatal diagnosis [15, 16].

The mean frequency of SLOS is 1:60,000 to 1:10,000 in live-births [17]. In Poland, however it may be even higher. As resulted from the studies conducted in our Department aiming to screen for carriership of commonest *DHCR7* mutation among neonates (p.W151X and p.V326L), the frequency of SLOS is about 1:3000 livebirths [18].

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Meanwhile, our subsequent, prospective studies which aimed to determine the SLOS prevelence during 2006-2008, resulted in significantly lower prevalence of the disease that would be expected – 1:866,273 ± 16,242, with estimated incidence of SLOS in Poland to be from 1:60,941 to 1:105,395 (1: 83,168 ± 22,227) livebirths [19]. One of the reason for this notable discrepancy between our previous carrier newborn screening and the prospective data may result from intrauterine death of affected fetuses. This hypothesis has been the subject of the studies presented in this paper.

Material and methods

Prenatal diagnosis toward Smith-Lemli-Opitz syndrome was conducted in the pregnancies with normal results of routine fetal karyotyping and min. 2 of the following ultrasound features:

- central nervous system: holoprosencephaly, agenesis/hypoplasia of corpus callosum,
- cleft palate,
- heart: atrioventricular septal defect,
- kidney: agenesis/hypoplasia, ectopia, cysts,
- external genitals: hypoplastic male, female with male karyotype (46,XY),
- limbs: polydactyly, abnormal position

and/or

- increased nuchal translucency (NT) > 3 mm during 11-13 week of gestation.

A total of 117 pregnant woman who provided informed consent entered this study, and fetal samples were obtained. In these cases we performed: 64 analyses in amniotic fluids and 53 in maternal urine.

In the first step, amniotic fluid samples were analyzed with gas chromatography/mass spectrometry (GC/MS) method to assess the concentration of 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC), as well as maternal urine samples to establish 7-dehydroestriol/estriol and 8-dehydropregnanetriol/pregnanetriol ratios [16].

Then, in pregnancies with confirmed elevated amount of these metabolites [with reference range (RR) for 7- and 8-dehydrocholesterol in amniotic fluid: 0-0,04 ug/ml; 7-dehydropregnanetriol / pregnanetriol: 0 – 0,014; and 8-dehydroestriol / estriol : 0-0,019 ratios in maternal urine sample] further molecular analyses were performed aimed to check for mutations in the *DHCR7* gene.

Results

In 4 cases out of 117 referred with suspicion of SLOS the syndrome was confirmed (diagnostic rate 0,03). Their clinical data are presented in Table I.

The mean age of diagnosed pregnancies was 19,6 weeks. The diagnoses of maternal urine were performed at 13, 18 and 22 weeks of gestations, while amniotic samples at 16 and 21 weeks. In 2 out of 4 pregnancies the increased nuchal translucency was observed (4,4 mm and 7,7 mm), another fetus was diagnosed with holoprosencephaly. In the last case no data are available for publication.

The diagnostic values of metabolites measured in maternal urine samples and(or) amniotic fluid using gas chromatography/mass spectrometry are presented in Table I.

Table I. Biochemical results (by GC/MS) in SLOS-positive pregnancies.

Patients	Prenatal features	Biochemical results			
		maternal urine (gestational age in weeks)		amniotic fluid (gestational age in weeks)	
I.P.	NT-7,7 mm	**18 weeks:**		**16 weeks:**	
		E3-358,2 ug/g creat. PT-420 ug/g creat. 7-DHPT/PT-0,41 8-DHPT/PT-0,57 8-DHE3/E3-0,32	N: 347 - 2267,5 N: 294,9 - 2235,1 N: 0 - 0,0140 N: 0 - 0,0140 N: 0 - 0,0190	CH-9,2 ug/ml 7-DHC-5,9 8-DHC-2,75	N: 16.4 +/- 4.8 N: 0-0,04 N: 0-0,04
K.P.-T.	no data (patient did not agree to share information)	**22 weeks:**		not analysed	
		E3-969,7 ug/g creat. PT-691,2 µg/g creat. 7-DHPT/PT-0,57 8-DHPT/PT-2,3 8-DHE3/E3-0,57	N: 2444 – 5472 N: 294 - 2335 N: 0 – 0,0140 N: 0 – 0,0140 N: 0-0,019		
M.S.	holoprosencephaly	not analysed		**21 weeks:**	
				CH-68,3 ug/ml 7-DHC-0,82 8-DHC-1,04	N: 20-100 N:0-0,04 N:0-0,04
M.K.	NT – 4,4 mm	**13 weeks:**			
		E3-76,3 ug/g creat. PT-385,5 ug/g creat. 7-DHPT/PT-0,035 8-DHPT/PT-0,054 8-DHE3/E3-0,12	N: 115,5-2207,9 N: 294,9-2235,1 N: 0 - 0,0140 N: 0 - 0,0140 N:0-0,019		

NT – nuchal translucency, E3 – estriol, PT – pregnanetriol, 7-DHPT/PT – 7-dehydropregnanetriol/pregnanetriol ratio, 8-DHPT/PT – 8-dehydropregnanetriol/pregnanetriol ratio, 8-DHE3/E3 – 8-dehydroestriol/estriol ratio, CH – cholesterol, 7-DHC – 7-dehydrocholestrol, 8-DHC – 8-dehydrocholesterol

Discussion

Our results concerning prenatal diagnosis of Smith, Lemli, Opitz syndrome based on suggestive ultrasound features (including increased NT and(or) specific malformations) show higher detection rate during 2-years period then our previous prospective data but similar to the most recent studies of Haas et al. [19, 20]. In the last cited paper, sterol concentrations in AF samples from 76 pregnancies with increased risk for SLOS, sent to the laboratory during 12 years (4 samples were further excluded because of limited clinical information) were analysed. In 6 fetuses, an abnormal sterol profile with increased 7-DHC and an increased ratio of 7-DHC/cholesterol was determined as diagnostic for SLOS. That gives the detection rate of 0,081%.

Two of the fetuses reported in AMJG, apart from multiple anatomical anomalies, also had intrauterine growth retardation (IUGR) and in one of these pregnancies MSuE3 (maternal serum unconjugated estriol) was reduced as well. The authors concluded that in cases with negative family history prenatal testing for SLOS should be perform when multiple malformations typical for SLOS are present, especially if there are additional IUGR or low MSuE3 [20]. Of note, in 3 of our 4 SLOS-positive pregnancies we found low concentration of estriol (E3) in maternal urine samples (Table I), which, with a very high probability, may have a diagnostic value, especially in karyotypically normal fetuses with specific malformations.

IUGR as the most common manifestation of SLOS was also noted in a study by Goldenberg. His group retrospectively evaluated 30 cases of SLOS for prenatal abnormalities and suggested to do sterol analysis in freezed AF sample when fetal karyotype is normal and another malformation are observed [21]. Moreover, there is also suggestion to test for SLOS simultaneously with chromosome analysis when MSuE3 is low, even if multiple anomalies are not detected on prenatal ultrasound examination.

As far as our proposed ultrasound anomalies predictive for SLOS are discussed, we like to point out toward holoprosencephaly as well as AVSD. These malformations, based on our knowledge of postnatal diagnosed patients, may be important key for prenatal diagnosis of Smith-Lemli-Opitz in fetuses with normal cytogenetic results. Another situations when SLOS should be considered during prenatal period are also mismatch of a cytogenetic test result and a fetal gender defined on the basis of ultrasound scans or external genital hypoplasia.

Our checklist, contrary to other publications, does not include intrauterine growth restriction (IUGR). From our experience, and available data on the SLOS cases diagnosed in our Department, the neonates are born mostly with normal birth parameters [22]. We do believe that increased NT could be more important prenatal marker (two out of 4 cases diagnosed in presented study had NT>3mm while none had IUGR).

Such a suggestion may be supported by results presented by Quelin et al. [23]. They report a series of 10 fetuses with molecularly proven SLOS (five with 46,XX and five with 46,XY) of whom three fetuses had increased nuchal translucency in the first trimester of pregnancy. Such a feature was also observed by Maymon et al. [24]. They proposed that some of fetal loss associated with SLOS may be related to nuchal oedema and subsequent fetal hydrops, reflecting the severity of the SLOS phenotype. Taking into account the increased NT during prenatal diagnosis of Smith-Lemli-Opitz syndrome seems to be the more reasonable that its measurement should be performed routinely in each pregnancy.

All data discussed above refer to SLOS suspicion and its verification in further, invasive tests (as chorionic villus sampling or amniocentesis). However, we like to draw attention to the possibility of performing noninvasive prenatal diagnostics of this condition. It is based on serial measurements of estriol (E3), pregnanetriol (PT), 7-dehydropregnanetriol (7DHPT) and 8-dehydroesteriol (8DHE3) concentrations in maternal urine samples obtained between 9 and 20 weeks of gestation [15, 16, 25].

As we can conclude based on cited publications as well as on our other unpublished data, steroid measurements in maternal random urine is a reliable method of prenatal diagnosis for SLOS. It may be the option for pregnant women who does not decide for invasive procedures or underwent Non-Invasive Fetal Trisomy Test (NIFT), which excluded these aberrations as a cause of fetal anomalies. Unfortunately non-invasive maternal urine testing for Smith-Lemli-Opitz syndrome is available only in a few laboratories worldwide.

Conclusions

Contrary to results of Haas et al., who found that multiple fetal malformations are not predictive for SLOS (with OR 3.13 CI95% [0.57; 17.05]) we suggest to perform biochemical sterol analyses or maternal urinary metabolites toward Smith-Lemli-Opitz in all pregnancies with suggestive ultrasound features (holoprosencephaly and(or) atrioventricular canal and(or) genital anomalies), especially when nuchal translucency is increased >3 mm, and after exclusion of chromosomal aberration in routine karyotyping or even arrayCGH. The GC/MS-based test for 7-and 8-dehydrocholesterols is relatively cheap, results are obtain during following 2 day, and may be performed in freezed AF sample.

Oświadczenie autorów

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