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Low Estriol Levels in the Maternal Marker Screen as a Predictor of X-Linked Adrenal Hypoplasia Congenita: Case Report

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SUMMARY

Introduction X-linked adrenal hypoplasia congenita (AHC) is a rare cause of adrenocortical insufficiency. Early postnatal diagnosis may prevent severe hypoglycemia, Addisonian crises and death. Low maternal estriol (E3) levels in the second trimester of pregnancy could indicate the possibility that the fetus suffers from a disorder that causes adrenal insufficiency. Suspicion is based on the fact that E3 originates from dehydroepiandrosterone (DHEA) synthesized in the fetal adrenals. In case of adrenal insufficiency, the impaired production of fetal DHEA leads to a subsequent reduction of E3 concentrations in maternal serum. There are only a few reports of AHC suspected prenatally due to low maternal E3 levels.

Case Outline We describe two brothers with adrenal insufficiency due to AHC. The older brother was admitted to the hospital at the age of 33 days due to failure to thrive, vomiting, and dehydration. Genetic analysis revealed a hemizygous mutation in DAX-1 gene, thus confirming the diagnosis of ACH. The same mutation was detected in his mother. In the second pregnancy, E3 concentrations were determined from maternal serum. Estriol levels during the second trimester were extremely low suggesting the diagnosis of AHC. The diagnosis was confirmed during the neonatal period by genetic testing, and replacement therapy was started at the age of 10 days. This boy never experienced an adverse episode such as hypoglycemia or adrenal crises.

Conclusion Since determination of E3 is a simple, sensitive, noninvasive and cheap method, its use as an obligatory prenatal screening test should be accepted as a standard practice in Serbia.

Keywords: free estriol in maternal serum; prenatal diagnosis; X-linked adrenal hypoplasia congenita

INTRODUCTION

X-linked adrenal hypoplasia congenita (AHC) is a rare cause of adrenocortical insufficiency. One per 12,500 newborns suffers from adrenal hypoplasia congenita. As in other X-linked disorders, the mother is almost always a healthy carrier and the risk for the male offspring being affected is 50%. Patients often present in infancy with failure to thrive or repeated vomiting, although presentations are very variable. Early diagnosis of AHC is extremely important, as death in the neonatal period appears to be common when the condition remains unrecognized. Prenatal diagnosis can be established by genetic testing or by low E3 concentrations in maternal serum in the second trimester of pregnancy.

During pregnancy, the main site of estrogen synthesis is the placenta, while estriol is its primary product. More than 90% of estriol in the maternal blood derives from the fetal adrenal cortex precursors C-19 DHEA [1]. The fetal adrenals synthesize dehydroepiandrosterone (DHEA) which undergoes hydroxylation into 16-hydroxydehydroepiandrosterone (16-OH DHEA) in the fetal liver. In the placenta, fetal 16-OH DHEA firstly becomes hydrolyzed under the influence of placental CYP17A1 enzyme. 16α-OH DHEA is then aromatized into estriol due to the activity of CYP19A1. Estriol flows into the bloodstream of the mother and fetus. In the liver, estriol is metabolized into conjugated forms, sulfates and glucuronides. Only unconjugated (free) estriol can be found in maternal serum. The conjugated form is excreted in urine (Figure 1). As the fetal adrenal cortex grows, the production of estriol and concentration in maternal serum grows too, as well as the amount excreted by urine. E3 is measurable from the 9th week of pregnancy and is present until the term. Decreased estriol concentration by more than 40% is considered significant suggesting fetal pathology. At the beginning of pregnancy, the synthesis of DHEA-SO4 in the fetal adrenal cortex is not dependent on the production of ACTH. Only from the second trimester of pregnancy fetal adrenal function becomes dependent of ACTH [2].

A significant number of different disorders that cause primary or secondary adrenal insufficiency as well as disorders in the placental function (such as aromatase deficiency) can lead to a decrease of E3 concentration in maternal serum. Low E3 levels are also found in pregnancies with trisomy 21, trisomy 13 and 18, and are used during prenatal screening for

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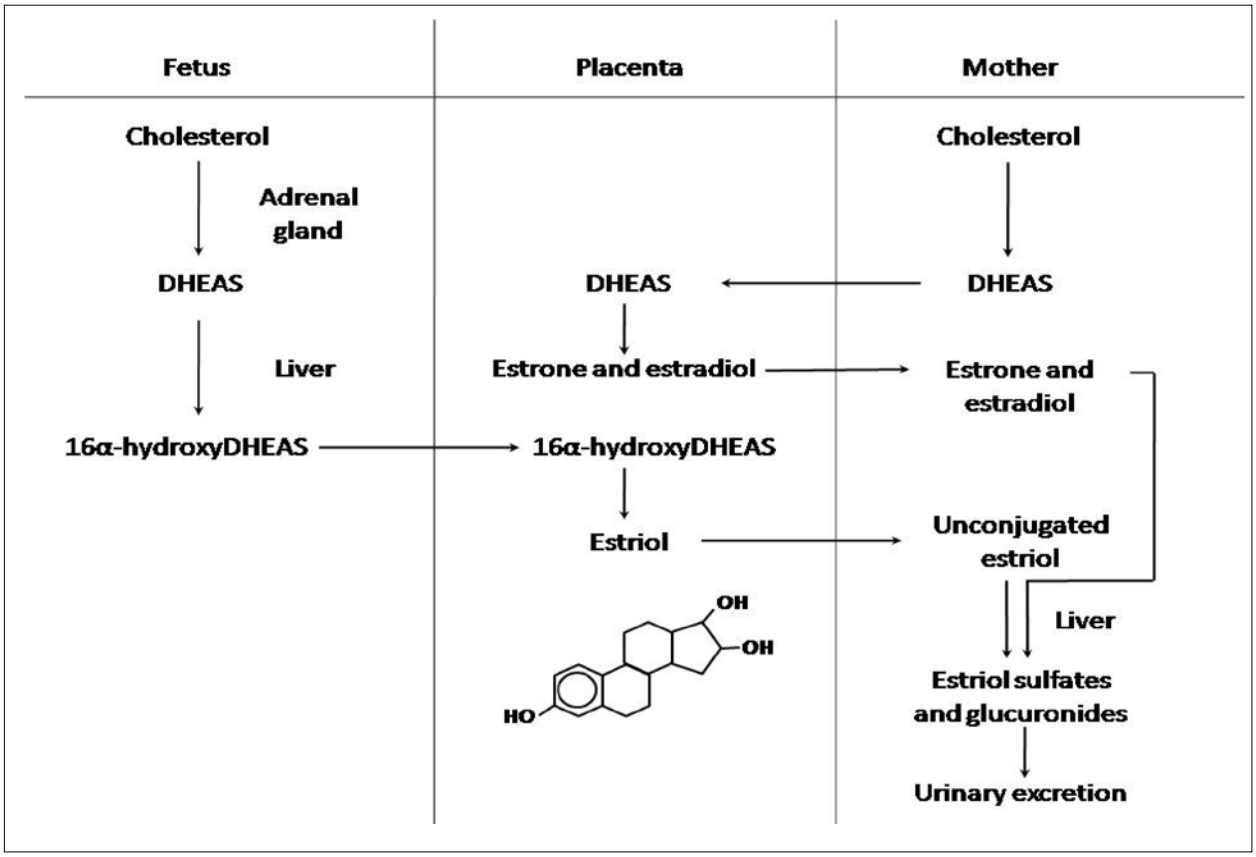

Figure 1. Fetal estriol synthesis

chromosomal anomalies during the second trimester of pregnancy [3]. In addition, the analysis of E3 concentrations from maternal serum can indicate fetal well-being [4]. E3 concentrations are monitored in high-risk pregnancies, such as the pregnancies of mothers with diabetes, hypertension and preeclampsia [5]. In the third trimester, a low E3 concentration can indicate fetal intrauterine growth restriction [6]. Fetal adrenal insufficiency should always be considered in the case of low E3 and a positive family history for AHC, especially if fetal sonography and karyotype are normal fetal sex male. If there is a prenatal suspicion of deficient fetal steroidogenesis, ACTH test and other investigations should be done in early neonatal period and diagnosis established in time to prevent severe hypoglycemia or adrenal crisis.

CASE REPORT

Healthy and unrelated parents were referred for genetic counseling because their first-born son was diagnosed with AHC. The 24-year-old mother was in the 13th week of her second pregnancy. She denied any drug ingestion during pregnancy or the occurrence of virilization. She was normotensive. There was a clinical suspicion of AHC in her firstborn at the age of one month. The diagnosis was confirmed by genetic testing. This boy (probant) was diagnosed with the mutation in *DAX1* gene [7]. Further investigations revealed that the mother was an asymptomatic carrier of a *DAX1* gene mutation. During her next pregnancy, due to a high risk of adrenal cortex insufficiency in the fetus, we planned appropriate prenatal testing. Since prenatal genetic testing was unavailable, we opted to determine E3 in maternal serum. The first trimester screening for chromosomal anomalies was performed; pregnancy associated plasma protein-A (PAPP-A) 1.6 mlU/ml (0.85 MoM) and free beta-human chorionic gonadotropin (free beta HCG) 32.6 ng/ml (0.90 MoM) were normal. Estriol concentration in the 23rd week of gestation was exceptionally low (0.7 ng/ml) (reference range 2.3 to 6.4 ng/ml). Estriol that was repeated in the 28th week of gestation was also extremely low (0.8 ng/ml; 2.3-7.7 ng/ml). Placental insufficiency as a potential cause of low E3 concentrations was excluded as human placental lactogen was normal in the 28th week. The ultrasound testing was satisfactory and the mother was pregnant with a male child. Due to a positive family history and normal fetal karyotype, it was highly likely that the low E3 levels were caused by fetal adrenal cortex insufficiency due to congenital adrenal hypoplasia. The delivery was without complications. At the age of 10 days, the following tests were carried out: K 6.2 mmol/l (4.1–5.6), Na 127 mmol/l (136–146), Cl 96 mmol/l (95–112), pH of blood 7.40, ACTH 1250 pg/ml (10–185), cortisol 74.8 nmol/l (190.4–579.4), plasma renin activity (PRA) >500 mIU/ml (2.8–39.9), 17-OH-progesterone 2.6 ng/ml (0.0–8.0) and DHEA-SO4 15.0 μg/dl (37–224). Other biochemical and ultrasonographic tests were within the normal range. Based on the extremely high level of ACTH and PRA, very low level of cortisol, low level of DHEA-SO4 and normal level of 17-OH-progesterone, the

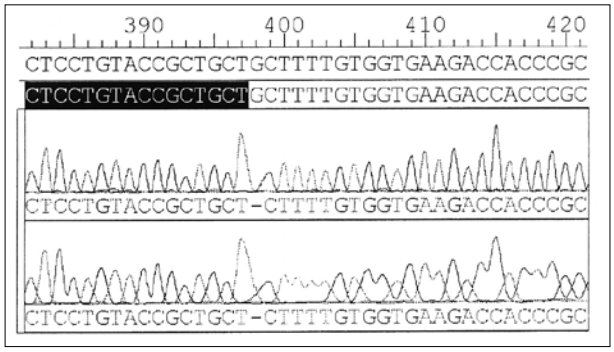

Figure 2. Representation of the child's DNA analysis, *DAX1* gene mutation on a chromosome Xp21 g398delG, C133fsX263

diagnosis of AHC was suspected and replacement therapy with hydrocortisone and fludrocortison was initiated. Genetic testing confirmed the same mutation in the DAX1 gene as it had been detected in the older brother. The genetic analysis showed a deletion of one base pair in exon of *DAX1* gene (g398delG) which could lead to a frameshift and a premature stop codon at amino acid position 263 of the DAX1 protein (Figure 2).

DISCUSSION

The analyses of free E3 levels, alpha-fetoprotein (AFP) and human chorionic gonadotropin (beta HCG) in the maternal serum have been performed as a part of the screening in the second trimester of pregnancy [8]. Although low E3 concentrations can be found in trisomy 13, 18 and 21, they are not as useful marker for chromosomopathies as PAPP-A protein and free beta HCG performed in the first trimester, which have a much higher sensitivity for chromosomopathies [9]. Recently, E3 has gained its significance as a biomarker for fetal and placental function. The importance of E3 in prenatal diagnosis of other diseases is currently being explored [10].

Very low E3 concentrations detected in cases of fetal death, serious placental insufficiency or congenital disorders of fetal adrenal cortex are of particular significance [11]. 1. Glucocorticoid therapy of pregnant women should be ruled out since dexamethasone can pass the placenta and suppress fetal adrenal cortex. 2. Steroid sulfatase deficiency occurs in X-linked ichthyosis. The deficient enzyme prevents the conversion of precursors into estriol. Therefore, E3 concentrations in the maternal serum are barely measurable, while elevated estriol precursors such as 16-OH-DHEA can be observed [12]. 3. Very low estriol is also registered in placental aromatase deficiency that results in androgen excess, virilization of the mother and the female fetus. 4. Diseases such as congenital lipoid adrenal hyperplasia, 17α-hydroxylase deficiency, P450 oxidoreductase deficiency and AHC, in which the production of DHEA-SO4 is extremely low or absent, can result in very low E3 concentrations [13]. 5. ACTH deficiency as a part of congenital hypopituitarism as well as the resistance to ACTH receptor can result in a reduced or absent synthesis of adrenal steroids and decreased level of E3 [14].

Table 1. Etiology of low level of fetal estriol in maternal serum

Fetus	Fetal death
	Adrenal hypoplasia
	SLOS
	LAH
	17 α-hydroxylase deficiency
	ACTH deficiency (hypopituitarism)
	Resistance to ACTH
	Trisomy 13, 18 and 21
Placenta	Insufficiency
	Steroid sulfatase deficiency
	Aromatase deficiency
Mother	Glucocorticoid therapy during pregnancy

SLOS – Smith-Lemli-Opitz syndrome; LAH – lipoid adrenal hyperplasia; ACTH – adrenocorticotropic hormone

Adrenal insufficiency can occur in disorders of cholesterol synthesis, such as rarely encountered Smith-Lemli-Opitz syndrome. Apart from adrenal cortex insufficiency, these patients are often diagnosed with multiple congenital anomalies and severe intellectual disability [15].

In daily practice, the interpretation of very low E3 concentrations from maternal serum is not simple. In such cases, a fetal karyotype should be obtained as a first step due to known risk for chromosomal anomalies. If the karyotype is normal, other causes of decreased estriol synthesis should be investigated. These causes can be divided into three groups: fetal, placental and maternal (Table 1). A history of hereditary diseases and consanguinity should be sought as well as any history of drug ingestion during pregnancy or the occurrence of maternal virilization during pregnancy. Meticulous fetal and placental ultrasound testing is of paramount importance. In the case of low E3 levels, workup must include measurement of DHEA-SO4 in the maternal serum and urine.

Low E3 concentrations can be indicative of decreased function of the fetal adrenal cortex. Other centers report similar experiences with prenatal biochemical screening [14, 15]. It enables the pediatric endocrinologist to directly assess the fetal adrenal cortex function shortly after birth. The early detection of fetal adrenal cortex insufficiency enables timely replacement therapy with glucocortidoids and, if necessary, mineralocortioids before life-threatening acute adrenal crisis becomes apparent.

If congenital disorders of the adrenal cortex remain unrecognized, there is a substantial risk of lethal outcome. Determining E3 in maternal serum is a noninvasive and cheap method which can reveal disorders in fetal steroidogenesis. Therefore we suggest that this kind of analysis should be introduced into screening during the second and third trimester of pregnancy in Serbia.

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For the purpose of this research all data were generated using Immulite 1000 analyser and Immulite 1000 Unconjugated Estriol reagent, both manufactured by Siemens Healthcare Diagnostics Inc.

REFERENCES

1. Fritz MA, Speroff L. Clinical Gynecologic Endocrinology and Infertility. Philadelphia: Lippincott Williams&Wilkins; 2010.
2. Buster J, Carson S. Endocrinology and diagnosis of pregnancy. In: Gabbe S, Niebyl J, Simpson J. Obstetrics – Normal and Problem Pregnancies. Philadelphia: Churchill Livingstone; 2002. p.3-36.
3. Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF. First and second trimester screening: detection of aneuploidies other than Down syndrome. Obstet Gynecol. 2007; 110:651-7.
4. Yaron Y, Cherry M, Kramer RL, Briten JE, Hallah M, Johnson MP, et al. Second trimester maternal serum marker screening: Maternal serum alpha-fetoprotein, beta human chorionic gonadotropin, estriol and their various combinations as predictors of pregnancy outcome. Am J Obstet Gynecol. 1999; 181:968-74.
5. Begum R. Diabetes in pregnancy and its management. Orion Medical Journal. 2008; 29:524-7.
6. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth : the role of the mother, placenta and fetus. Endocr Rev. 2006; 27:141-69.
7. Reutens A, Achermann J, Ito M, Gu W, Habiby R, Donohoue P, et al. Clinical and functional effects of mutations in the DAX-1 gene in patients with adrenal hypoplasia congenita. J Clin Endocrinol Metab. 1999; 84:504-11.
8. Canick JA, MacRae AR. Second trimester serum markers. Semin Perinatol. 2005; 29:203-8.
9. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening of chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-step clinic: a review of three years prospective experience. Br J Obstet Gynaecol. 2003; 110:281-6.
10. Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA. Obstetrical complications associated with abnormal maternal serum markers analytes. J Obstet Gynaecol Can. 2008; 30:918-49.
11. Schleifer RA, Bradley LA, Richards DS, Ponting NR. Pregnancy outcome for women with very low levels of maternal serum unconjugated estriol on second trimester-screening. Am J Obstet Gynecol. 1995; 173:1152-6.
12. Langlois S, Armstrong L, Gall K, Livinghton J. Steroid sulfatase deficiency and contiguous gene deletion syndrome amongst pregnant patients with low serum unconjugated estriol. Prenat Diagn. 2009; 29:966-74.
13. Jean A, Mausukhami M, Olerfield SE, Fennoy I, Nakamoto J, Atwan M. Prenatal diagnosis of congenital lipoid adrenal hyperplasia (CLAH) by estriol amniotic fluid analysis and molecular genetic testing. Prenat Diagn. 2008; 28:11-4.
14. Weintrob N, Drouin J, Vallete KS, Taub E. Low Estriol levels in the maternal triple marker screen as a predictor of isolated adrenocorticotropic hormone deficiency caused by a new mutation in the TPIT gene. Pediatrics. 2006; 117:322-7.
15. Shackleton C, Roitman E, Kratz L, Kelley R. Dehydro-oestriol and dehydropregnanetriol are candidate analytes for prenatal diagnosis of Smith-Lemli-Opitz syndrome. Prenat Diagn. 2001; 21:207-12.

Низак ниво естриола у серуму мајке одређен у оквиру пренаталног скрининга као предиктор X-везане конгениталне адреналне хипоплазије – приказ болесника

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КРАТАК САДРЖАЈ

Увод *X*-везана конгенитална адреналнa хипоплазија је редак узрок адренокортикалне инсуфицијенције. Рана постнатална дијагноза може спречити тешку хипогликемију, Адисонову кризу и смрт. Низак ниво естриола (Е3) у серуму мајке у другом триместру трудноће може да укаже на адреналну инсуфицијенцију код плода. У поремећајима који доводе до адреналне инсуфицијенције производња *DHEA* је смањена, што за последицу има снижен ниво Е3 у серуму мајке. Досад је објављено свега неколико радова о сумњи на *X*-везану конгениталну адреналну хипоплазију у пренаталном добу због изузетно ниског нивоа Е3 у серуму мајке.

Приказ болесника Приказана су два брата с адреналном инсуфицијенцијом као последицом *X*-везане конгениталне адреналне хипоплазије. Старији брат је болнички лечен као одојче узраста од 33 дана због ненапредовања, повраћања и дехидратације. Генетичко испитивање је открило мутацију у гену *DAX-1* и потврдило *X*-везану конгениталну адреналну хипоплазију. Иста мутација је откривена код мајке. У следећој трудноћи ниво естриола измерен у другом триместру је био изузетно низак, што је указивало на могућност конгениталне адреналне хипоплазије. Дијагноза је потврђена генетичким испитивањем новорођенчета. Супституциона терапија је започета у узрасту од десет дана, чиме су спречене хипогликемија и адренална криза.

Закључак Будући да је одређивање Е3 у серуму мајке једноставна, сензитивна, неинвазивна и јефтина метода, препоручујемо да се уведе као метода скрининга у трудноћи.

Кључне речи: естриол у серуму мајке; пренатална дијагноза; *X*-везана конгенитална адренална хипоплазија

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