How to Manage Low Estriol Levels in Pregnancies, One Center Experience

Gebeliklerde Düşük Östriyol Düzeyleri Nasıl Yönetilir, Tek Merkez Deneyimi

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ABSTRACT

Objective: Low estriol (uE3) levels in the second-trimester screening for Down syndrome may be the result of fetal demise, congenital abnormalities, or some genetic hormonal disorders of the fetus. Although X-linked ichthyosis, a microdeletion syndrome with mild ichthyosis, which causes steroid sulfatase (STS) deficiency, is the most common genetic cause, second-trimester screening tests calculate the risk for a less common and severe disorder known as the Smith Lemli Opitz syndrome (SLOS). We aimed to investigate the outcomes of pregnancies with low uE3 levels in Down syndrome screening and emphasize the high prevalence of STS deficiency instead of SLOS in such cases.

Methods: Fifteen pregnancies with very low uE3 levels and high risk for trisomy and/or SLOS in screening tests were evaluated and tested for STS deficiency and SLOS.

Results: Seven of the pregnancies had STS microdeletion syndrome, while additional two cases were supposed to have STS gene mutation according to family and/or postnatal history. Although one fetal death was recorded, no chromosomal abnormality, SLOS, or congenital malformation was recorded in our series.

Conclusions: SLOS is a very severe and rare syndrome. The risk estimation for SLOS in screening tests causes stress for pregnant women and healthcare givers. We recommend the addition of risk estimation for STS deficiency when a low uE3 level is detected in the screening test.

Keywords: Genetic counseling, low unconjugated estriol, maternal serum screening, prenatal cytogenetics, Smith Lemli Opitz syndrome, steroid sulfatase deficiency

ÖZ

Amaç: İkinci trimester Down sendromu tarama testlerindeki düşük östriyol (uE3) seviyesi, fetal ölüm, konjenital anormallikler veya fetüsün çeşitli genetik hormonal bozukluklarından kaynaklanabilir. Steroid sülfataz (STS) eksikliğine neden olan ve hafif iktiyozla seyreden bir mikrodelesyon sendromu olan X'e bağlı iktiyoz en yaygın genetik neden olmasına rağmen, ikinci trimester tarama testleri daha az yaygın ve daha şiddetli bir hastalık olan Smith Lemli Opitz Sendromu (SLOS) için risk hesaplamaktadır. Down sendromu taramasında uE3 düzeyi düşük olan gebeliklerin sonuçlarını araştırmayı ve bu gibi durumlarda SLOS yerine STS eksikliğinin yüksek prevalansını vurgulamayı amaçladık.

Yöntemler: Tarama testlerinde uE3 seviyeleri çok düşük olan ve trizomi ve/veya SLOS açısından yüksek risk taşıyan on beş gebelik STS eksikliği ve SLOS açısından değerlendirilmiş ve test edilmiştir.

Bulgular: Gebeliklerin yedisinde STS mikrodelesyon sendromu bulunurken, ek iki olguda aile ve/veya doğum sonrası öyküye dayanarak STS gen mutasyonu düşünüldü. Bir fetal ölüm tespit edildi. Ek kromozom anomalisi, SLOS veya konjenital malformasyon tespit edilmedi.

Sonuçlar: SLOS çok ağır seyreden ve nadir görülen bir sendromdur. Tarama testlerinde SLOS için risk tahmini hamileler ve sağlık çalışanları için strese neden olmaktadır. Anksiyeteyi önlemek için tarama testlerinde düşük bir uE3 seviyesi tespit edildiğinde STS eksikliği için risk tahmininin eklenmesini öneririz.

Anahtar kelimeler: Genetik danışmanlık, düşük konjuge olmayan östriyol, maternal serum taraması, prenatal sitogenetik, Smith Lemli Opitz sendromu, steroid sülfataz eksikliği

INTRODUCTION

As the prenatal screening methods evolve, the antenatal first and second-trimester screening for Down syndrome becomes outdated. Nowadays, non-invasive cell-free fetal DNA screening has emerged. However, in many parts of the world, due to its high cost and the limited number of laboratories performing the test, it is still not a part of the general population screening during pregnancy. Besides, both first and second-trimester screening for Down syndrome provides additional information about disorders other than aneuploidies.

Second-trimester screening for serum markers is a useful tool for estimating the risk for most common trisomies (trisomy 21,13, and 18) and open neural tube defects[1]. Besides, they can suggest the risk for various conditions concerning maternal and fetal health[2,3].

Low estriol (uE3) levels below half or ¾th of the normal levels during the second or third trimester of pregnancies are usually associated with fetal growth restriction, fetal demise or loss, and large neural tube defects, such as anencephaly and several congenital malformations (Table 1)[2-4]. While accompanying the abnormal levels of other screen parameters, it generally indicates the risk for aneuploidies. Isolated very low levels of uE3 are usually associated with fetal or placental hormonal abnormalities[5]. In different studies, <0.25 to <0.10 MoM or <0.25 to <0.15 ng/mL are estimated as the cut-off levels for very low-level definition[3,5-9].

The most common hormonal deficiencies include steroid sulfatase (STS) deficiency (X-linked recessive ichthyosis, OMIM #308100), Smith Lemli Opitz syndrome (SLOS, OMIM #270400), placental aromatase deficiency (OMIM #613546), fetal isolated adrenocorticotropic hormone deficiency (OMIM #201400), primary fetal adrenal insufficiency due to congenital adrenal hypoplasia (OMIM #300200), lipoid adrenal hyperplasia (OMIM #201710), and 17 alpha-hydroxylase deficiency (OMIM #202110) (Table 1)[3,4,6,10-13].

SLOS is an autosomal recessive disorder of cholesterol metabolism, which causes very low uE3 levels in pregnancies. SLOS risk estimation has been part of the second-trimester screening tests for over a decade[14,15]. Deficiency of 7-dehydrocholesterol reductase [sterol delta-7-reductase *(DHCR7)*, OMIM #602858] causes SLOS, which leads to multiple congenital malformations of the limbs and digits, eyes, oral region, internal organ systems, microcephaly, mental retardation, and hypogonadism[16-18]. The incidence of SLOS is approximately 1 in 20,000 to 30,000 births[19]. Deficiency of the enzyme

causes high levels of 7-dehydrocholesterol and low levels of cholesterol in the serum of the patients[18]. In the affected pregnancies, 7-dehydrocholesterol levels of amniotic fluid and chorion villus are increased, while cholesterol levels, as a consequence of estriol levels, are decreased in the same tissues[20]. *DHCR7* gene sequencing detects mutation in 96.6% of the cases.

An X-linked recessive ichthyosis is a common form of ichthyosis caused by mutation of the *STS* gene (OMIM #300747) encoding for the enzyme STS on chromosome Xp22.3. Its incidence ranges between 1 in 1,300 to 1 in 6,000 males worldwide. It is inherited in an X-linked recessive manner, which exclusively makes it a disorder in males. Patients show classical scaly ichthyotic plaques in the first months of life[21-24]. In the pregnancies of affected males, placental STS deficiency causes low uE3 levels. This could cause a delay in the onset of labor, post maturity, sudden fetal death, and relative refractoriness to the oxytocic agents during labor[21]. Most patients (90%) have a deletion of the *STS* gene as a part of the chromosomal microdeletion of Xp22.3. In more than

Table 1. Causes of low uE3 levels.
Trisomy 13,18, 21 - 45,X - triploidy and rare chromosome abnormalities
Fetal death
Fetal growth retardation
Major congenital malformations
Anencephaly
Holoprosencephaly
Cardiopulmonary malformations
Gastroschisis
Brain/skeletal/genital malformations
Multiple malformations
Preterm labor risk
X-linked ichthyosis (STS deficiency) and/or contiguous gene syndrome
Smith Lemli Opitz syndrome
Placental aromatase deficiency
Primary fetal adrenal insufficiency
Lipoid adrenal hyperplasia
17 alpha-hydroxylase deficiency
Congenital adrenal hypoplasia
Secondary fetal adrenal insufficiency
Exogenous maternal corticosteroid treatment
Congenital fetal hypopituitarism (isolated fetal ACTH deficiency)
STS: Steroid sulfatase, ACTH: Adrenocorticotropic hormone, uE3: Estriol

two-thirds of the cases, it is familial, as it is inherited from a carrier mother to her son. In 5% to 10% of the cases, other mutations (point mutations or gene deletions-duplications) of the *STS* gene are considered the cause of the disorder[24]. Since most of the cases are due to the microdeletion of the Xp22.3 chromosomal region, fluorescent *in situ* hybridization (FISH) analysis and array comparative genomic hybridization (aCGH) are the most common tools used to detect the disorder. In this study, we aimed to investigate subjects who have low uE3 levels in their second-trimester screening tests. We reviewed our records and documented the features and outcomes of our cases with very low uE3 levels, as well as the SLOS risk in the second-trimester screening tests.

MATERIALS and METHODS

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Istanbul Kanuni Sultan Suleyman Training and Research Hospital (decision no: KAEK/2018.5.13). Written informed consent was obtained from all patients.

Patients: We performed a retrospective study of patients who applied to the Prenatal Genetics Unit with an abnormal second-trimester screening test. We evaluated pedigrees, familial, and individual histories of all cases.

We evaluated the results of STS deficiency deletion syndrome via FISH analysis, *DHRC7* gene analysis, and chromosomal analysis of peripheral blood lymphocytes and amniocytes of the subjects. Records of detailed prenatal ultrasonography (USG) of all cases were evaluated. Pregnancy outcomes were followed-up via phone calls and office visits.

Chromosomal Karyotyping: Peripheral blood samples were collected from patients and inoculated into RPMI 1640 medium. The cultures were incubated at 37 °C for 72 hours and then treated with colcemid.

Amniotic fluid samples (20 mL amniotic fluid) were obtained from patients and cultured with three different mediums in three different flasks. After seven days, the flasks were examined under an inverted microscope.

Cultured cells obtained from the peripheral blood or amniotic fluid were harvested and fixed onto slides. Giemsa (Leishman) was used for the G-banding of the metaphase chromosomes and, at least, 20 metaphase cells were counted and five cells were analyzed for each patient.

FISH: FISH was performed on the metaphase spread from the peripheral blood or amniotic fluid according to the manufacturer's instructions using Vysis STS Deficiency LSI STS Spectrum Orange/CEP X Spectrum Green Probes, Abbott. At least, 60 interphase nuclei were analyzed for each FISH assay (Figure 1).

***DHCR7* Gene Sequencing:** The spin-column method was used for the extraction of genomic DNA (PureLink® Genomic DNA Kits) from peripheral blood lymphocytes and cultured amniocytes. The concentration of the DNA samples was measured by spectrophotometry (Nanodrop ND-1000) and the DNA samples were stored at -20 °C until further use. We amplified and sequenced all the exons and exon-intron boundaries of the *DHCR7* gene (NM_001360.3) using the primers listed in Table 2. Polymerase chain reaction (PCR) was performed in a final volume of 50 mL, which contains 17.5 μL of distilled sterile water, 10X buffer solution, 1.5 μL of Taq polymerase, 10 mmoL of dNTPs, 25 mmoL of MgCl2, 150 ng of genomic DNA, and 10 pmol of forward/reverse primer. The temperature of the cycling conditions is as follows: 95 °C (5 min, Taq polymerase activation); 95 °C (30 sec, denaturation); 35 cycles of 30 sec at 95 °C, annealing for 30 sec at 58 °C, and extension for 30 sec at 72 °C; the final extension was done at 72 °C (10 min) (Applied veriti). The amplified PCR product was confirmed by agarose gel electrophoresis and then sequenced with APPLIED 3730 DNA Analyzer (48-capillary) automated DNA sequencer (according to the manufacturer's protocol). The raw

Table 2:. Primers used for *DHCR7* gene amplification and sequencing.

| Primer	Forward	Reverse
DHCR7_3	GGTGGATGCAACAGGGAAAGGTGG	AGGCTGGAAAGCTCTGAG
DHCR7_4	ATCCTCTCCGACCTGGAACT	CACGGGGTTTTGCTCCTAT
DHCR7_5	GTGACTGGGAGGGACCTGT	AATGGTGGTCTCTGCATGGT
DHCR7_6	ACGAGATGCAGAACCAAAGG	TCCAAAGAAAGAGGCAATGG
DHCR7_7	GCTGAATGCAAAGCAAAGTG	GCTTCCTTCACCAAGTGCTC
DHCR7_8	AATGTATCCCTTCCCCTTGG	AGGAGGCAGGAATGAAGAGG
DHCR7_9	CACAGACAGGTAGAAGGCAGGT	GGCAAAAGCAAGGAACAGAG
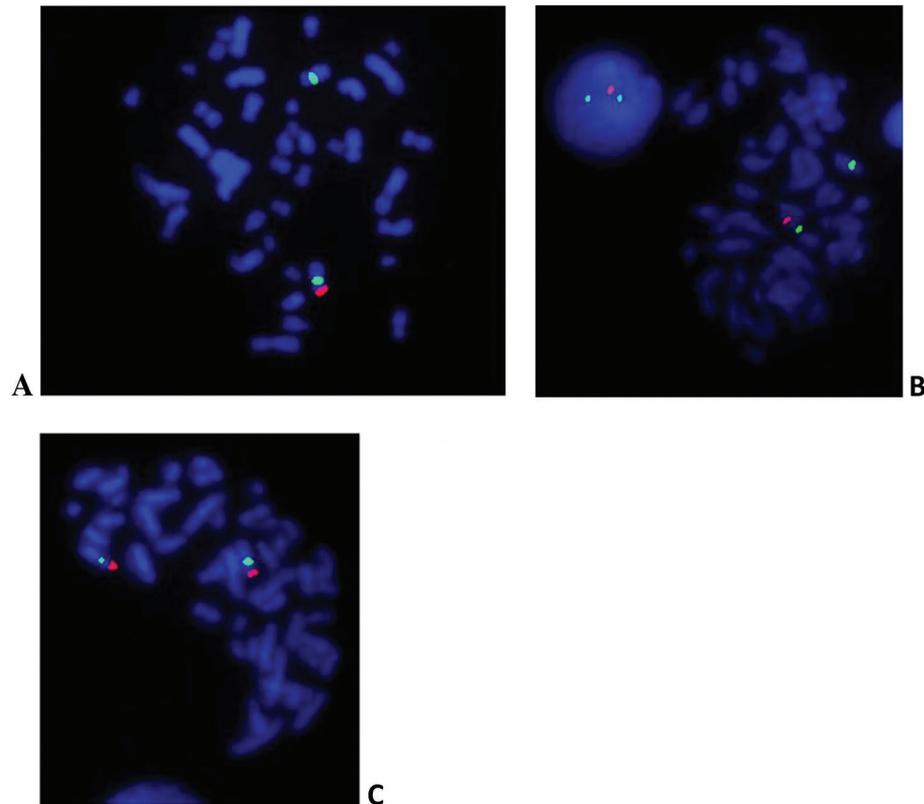
Figure 1. STS FISH images: Figures A and B show deletion with two green and one red signal. Figure C shows a normal image with two red and two green signals.

sequence data were analyzed using the CHROMAS analysis program.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel version 2019. Mean, median, minimum, maximum, and percentage values were obtained using this software.

RESULTS

Fifteen pregnant women with very low uE3 levels, with or without high SLOS risk in the second-trimester screening tests, were evaluated. The age of the pregnant women was in the range of 20-42 years (mean age: 28.3, median age: 28). All second-trimester screening test results showed a high risk for Down and/or Trisomy 18 syndromes. In addition, in eight of the cases, a high risk for SLOS was recorded (Table 3). We contacted all the cases after the delivery of their baby, except two (Table 3).

The uE3 levels were in the range of 0.04-0.22 mom. In seven of the cases, there was a positive family history of ichthyosis with an X-linked recessive inheritance model (Table 3).

In five of these cases with a positive family history of X-linked recessive inherited ichthyosis, STS FISH analysis showed heterozygous deletion (cases 3, 4, 8, 11, and 14). In two of them, STS FISH was normal, thus, we suspected that they probably carry *STS* gene mutations (cases 5 and 6) (Figure 1). In two subjects, STS FISH analysis revealed deletion without a family history (cases 2 and 9). Thus, a total of seven pregnant women carried the heterozygous deletion on *STS* gene locus. Nine of the pregnancies were probably affected by X-linked ichthyosis (Table 3).

One of the pregnancies resulted in fetal death (male fetus) in the 8th month of pregnancy and no abnormality or growth retardation in the fetus was observed (case 12). The family history was unremarkable, except for the consanguinity between parents. Maternal age was 42 and STS FISH and *DHCR7* gene analyses of the mother were normal and no fetal genetic workup was done. We were not able to analyze the *DHCR7* gene intragenic big deletions and *STS* gene mutations of the mothers.

Seven of the families were approved for amniocentesis. All fetal karyotypes were normal. FISH analysis of three showed heterozygous deletion (the mothers had heterozygous deletion also) (Figure 1). In three of them,

there was no deletion similar to that of the mothers of the fetuses. One of the prenatal FISH analyses was uninformative (Table 3).

Four of the babies were evaluated after birth. All the babies had ichthyosis on examination (Figure 2). Three were babies born by microdeletion carrier mothers. The prenatal FISH analysis was positive for ichthyosis in two of the cases but uninformative in one case that had a microdeletion according to the postnatal FISH analysis. One was the baby born by an STS FISH normal mother with positive family history; his FISH analysis after birth was normal but he had ichthyosis. We were not able to conduct an STS gene analysis (Table 3, 4).

We evaluated nine cases via phone calls after birth during the first three years of their babies' lives. To our knowledge, the youngest was 6 months old and was affected (case 3). All babies were male, six of whom were healthy babies (one had atopic dermatitis) born at term with normal birth weight following uneventful pregnancies. One was the son of a mother with family history of X-linked ichthyosis and normal FISH result. The STS FISH analysis and a family history of the other mothers were normal (Table 4).

Two of the cases had babies with ichthyosis according to their mothers' descriptions. One was born at the 41st week via caesarian section (C/S) due to delayed delivery, no response to induction of delivery, and fetal distress;

the other was born at term via C/S due to repeated C/S (the first child was also affected and his birth was delayed, thus, he was born via C/S due to fetal distress). Mothers of both babies were carrying heterozygous STS deletion. (Table 4). Parental and/or fetal *DHCR7* gene analyses of five cases ware normal (Table 3).

Prenatal USG was normal in all the cases. The cases we could not contact after pregnancy were uneventful

Figure 2. Ichthyotic plaques on the extensor surfaces of the lower extremities of one of the babies.

Table 3. Test results of the pregnancies.								
Patients	Maternal age (year)	Gestational week	uE3 (mom)	uE3 (ng/mL)	SLOS risk in screening	STS FISH* (maternal)	STS FISH* (AS)	*DHCR7* gene analysis
1	26	19	0.02	0.03	+	-	-	N
2	20	20	0.03	0.04	+	+	NA	N
3	27	19+2	0.04	NA	+	+	+	NA
4	33	19+	0,07	NA	NA	+	NI	NA
5	22	18	0.07	0.09	+	-	NA	NA
6	20	17	0.08	NA	+	-	NA	NA
7	33	20	0.09	NA	NA	-	NA	NA
8	29	18+3	0.09	NA	+	+	+	NA
9	28	17+4	0.11	NA	NA	+	+	NA
10	36	16+	0.14	0.4	+	-	-	N
11	33	17	0.14	<0.07	NA	+	NA	NA
12	42	16	0.17	<0.07	NA	-	NA	N
13	27	18+1	0.18	NA	+	-	-	N
14	20	17+1	0.2	NA	+	+	NA	NA
15	29	20+3	0.22	NA	NA	-	-	NA

*"-" means STS FISH analysis is normal, "+" means there is heterozygous microdeletion according to STS FISH analysis. N: Normal, NA: Not available, NI: Non-informative, STS: Steroid sulfatase, SLOS: Smith Lemli Opitz syndrome, FISH: Fluorescent *in situ* hybridization, uE3: Estriol

Table 4. Test results and outcomes of pregnancies.									
Patients	uE3 (mom)	SLOS risk in screening	STS FISH* (maternal)	STS FISH* (fetal)	Family history of ichthyosis	Pregnancy course	Type of delivery	Fetal sex	Postnatal phenotype
1	0.02	+	-	-	-	N	NVB	M	N
2	0.03	+	+	NA	-	N	NA	M	NA
3	0.04	+	+	+	+	N	NVB	M	Ichthyosis
4	0.07	NA	+	NI	+	N	NVB	M	Ichthyosis
5	0.07	+	-	NA	+	N	NVB	M	N
6	0.08	+	-	NA	+	N	NVB	M	Ichthyosis + cryptorchidism
7	0.09	NA	-	NA	-	N	NVB	M	N
8	0.09	+	+	+	+	N	NVB	M	Ichthyosis
9	0.11	NA	+	+	-	N	NA	M	NA
10	0.14	+	-	-	-	N	NVB	M	N
11	0.14	NA	+	NA	+	N	C/S[b]	M	Ichthyosis
12	0.17	NA	-	NA	-	Sudden fetal death	-	M	-
13	0.18	+	-	-	-	N	C/S[b]	M	N
14	0.2	+	+	NA	+	Oligohydramnios	C/S[c]	M	Ichthyosis
15	0.22	NA	-	-	-	Fetal anomaly[a]	NVB	M	Atopic dermatitis

NA: Not available, N: Normal, NI: Non-informative, NVB: Normal vaginal birth, C/S: Cesarian section, M: Male, STS: Steroid sulfatase, FISH: Fluorescent *in situ* hybridization, uE3: Estriol, SLOS: Smith Lemli Opitz syndrome, [a]Mild fetal renal pyelectasis, [b]C/S due to previous C/S history, [c]C/S due to delayed birth, *: "–" means STS FISH analysis is normal, "+" means there is heterozygous microdeletion according to STS FISH analysis

pregnancies of male fetuses according to prenatal USG and follow-up records (cases 2 and 9). The mothers of these two cases had heterozygous STS microdeletion and one also had heterozygous STS microdeletion of amniocytes (Table 4).

DISCUSSION

In previous series, an increased incidence of perinatal complications in the presence of very low uE3 levels was reported. The most common causes observed were fetal death and fetal growth retardation. While in some of the cases the cause remained undetermined, the most common genetic cause reported was X-linked ichthyosis; the second most common genetic cause was abnormal karyotype of the fetus. SLOS was very rare[3,4,6-9,25].

X-linked recessive ichthyosis, also known as STS deficiency syndrome, is a microdeletion syndrome is found in 90% of patients. Since it results from a microdeletion, it carries the risk of transforming into a contiguous gene deletion syndrome when the deletion gets large enough to encompass adjacent genes. According to the adjacent genes affected, the patient may present with additional features, such as mental retardation, Kallmann syndrome, ocular albinism, X-linked chondrodysplasia punctata, and SHOX gene-related short stature[26]. In most of the familial cases, the deletion inherited remains unchanged as in most other inherited forms of microdeletions; however, in cases with a *de novo* deletion without family history and a carrier mother, there is up to 10% risk of contiguous gene deletion syndrome[27]. In our patients, according to the information we obtained, there were no affected families or babies from a contiguous gene deletion. In 5% to 10% of the instances, the syndrome was the result of *STS* gene mutations. Two of our patients had no deletion in the STS FISH analysis, despite having a family history of male ichthyosis and one of the babies had ichthyosis in the first months of life. Both cases probably had *STS* gene mutations; unfortunately, we could not test them.

SLOS is a very grave syndrome with multiple anomalies. Since it causes a decreased synthesis of 7-dehydrocholesterol,

the precursor of uE3 synthesis, Bradley et al.[14] and Palomaki et al.[15] suggested a new screening method for SLOS as a part of the second-trimester screening test using estriol level as a biomarker. Both articles were prepared according to the antenatal values of proven cases of SLOS. According to Bradley et al.[14], 12 of the 26 SLOS cases had uE3≤0.20 mom; however, uE3 levels as high as 0.65 mom were observed and 24 of the 26 cases had uE3 levels below 0.52 mom (below the 5th centile for the author). According to Palomaki et al.[15], the mean mom value of uE3 for SLOS pregnancies was 0.21 (standard deviation: -0.6778). Bradley et al.[14] suggested that as the screening for SLOS is added to the second-trimester screening test, more SLOS cases would be diagnosed antenatally and that it would be cost effective to diagnose such a severe disease antenatally. However, according to Palomaki et al.'s[15] data, only 1 in 90 positive tests would be SLOS, based on best calculations. In several series revising the outcomes of the pregnancies with low and very low uE3 levels, the numbers of SLOS cases detected are very low[3,4,6]. In Minsart et al.'s[3] review of 9 series, only the series of Schoen et al.[6] diagnosed two cases. In Craig et al.'s[4] study interpreting the outcomes of SLOS-positive women out of a total of 777,088 screened women, only 4 cases of SLOS were diagnosed in a total of 2,018 screened positive women. As long as it is an autosomal recessive disorder and since its incidence is higher in communities with a high consanguineous marriage ratio as ours, we rarely come across SLOS in clinical practice. We did not diagnose any SLOS in our series. Since SLOS causes many fetal anomalies, it should be kept in mind that detailed ultrasonographic findings suggestive of it, such as brain malformations, polydactyly, oral cavity malformations, and ambiguous genitalia accompanying very low uE3 levels, should raise the need for SLOS screening. Normal USG significantly reduces the risk of the disease.

In most of the series reported, the ratio of male to female fetuses was high among viable pregnancies. It was related to the high prevalence of STS deficiency in such pregnancies[4,6,25]. In all series including STS deficiency into their study protocol, STS deficiency was the most common genetic cause of low uE3 level and positive SLOS screening test. In addition, studies strongly suggest that pregnancies with positive SLOS screening may have severe outcomes, such as fetal death, growth retardation, chromosomal abnormalities, anencephaly, holoprosencephaly, and cardiopulmonary or gastrointestinal tract malformations, which are usually more common than SLOS[3,4,6-9,25,28].

As outlined by various studies, in the absence of additional fetal and/or maternal disorders, X-linked STS deficiency is the most common cause of very low levels of uE3. Similarly, in our series, in seven of the 15 cases, the mothers had STS heterozygous microdeletions, while two more cases were supposed to have STS pathogenic gene variations (single nucleotide variants) according to the family history and/or phenotype of the child born (Table 4).

Since most of the cases are familial, the deletion inherited would consequently remain unchanged. Therefore, we questioned the family and personal history of the mothers and their previous labors. We conducted STS deletion FISH testing to detect the carrier status of the mothers and, as a result, we had information about the carrier status of the mothers. We had a presumption about the reason for the low uE3 level and foresight about the risk for delayed and complicated labor. In addition, once the mother was diagnosed as a carrier, especially if the family history is positive for isolated X-linked ichthyosis, we had the chance to inform the family about the high probability of having a baby boy with the same ichthyosis phenotype that is not complicated with a contiguous gene deletion syndrome. We also had the chance to inform the family about the decreased risk of having a child with another aneuploidy and SLOS (if the USG is normal), as well as the decreased need for more invasive diagnostic tests, such as amniocentesis. It is possible to re-evaluate the screening test for aneuploidy risk estimation with serum analyte levels that exclude estriol. Although the detection of the carrier status of mothers did not warrant the absence of another aneuploidy of the fetus, we informed the families about the option of having invasive aneuploidy testing.

Four of the eight STS microdeletion non-carriers had an STS FISH analysis of the amniotic fluid and all results were normal. None of the fetuses had *de novo* microdeletion (Table 3 and Table 4). Since the risk of contiguous gene deletion syndrome may be as high as 10% in de novo deletions, there is a need for additional tests, such as aCGH testing, in those cases. Thus, considering that we had no de novo deletion in our series, the need for more detailed testing, such as aCGH, was miscellaneous. We contacted the families after the birth of the babies and before the submission of this article to ask for additional symptoms suggestive of a contiguous gene deletion syndrome. None of the children had additional symptoms or features suggestive of a contiguous gene deletion syndrome. Although aCGH is the test of choice for high risk pregnancies with USG markers and abnormalities in recent years, it is more expensive and time-consuming. Also, the excess data obtained as various variants of unknown significance is

usually confusing compared to FISH analysis. We still do not prefer the use of aCGH in high risk serum screening tests[29].

All the fetuses in our study were male. We suppose that this male sex predominance was due to the STS genotype in most of our cases as in previous studies. STS deletion syndrome is an X-linked recessive disorder; thus, it mainly affects males and pregnancies of male fetuses, except in rare cases of homozygous females or skewed X inactivation cases in which the females are also affected. Although all fetuses in our series were male, we did not make a gender selection via USG before we tested the mothers for STS deletion. In general practice, gender identification via antenatal USG may be useful in risk estimation for X-linked disorders, such as STS deficiency.

There was no chromosomal abnormality or other major congenital abnormalities in our study. We suppose that this is because most of the cases with chromosomal abnormalities or major congenital abnormalities show abnormalities on fetal USG before their second-trimester screening for Down syndrome; thus, these cases usually present to us with abnormal USG results without screening risks and were, therefore, excluded in our series. Therefore, it can be said that if a detailed pregnancy evaluation with USG is performed before the second-trimester screening, further workup or time delay may be prevented.

Although there was no growth retardation or other abnormalities according to USG and genetic tests of the mother, one pregnancy ended with sudden fetal death at the 8th month of pregnancy. The maternal age was 42 but there was no abnormality other than the second-trimester screening result and the very low uE3 value. Thus, abnormal second-trimester screening, especially very low uE3 levels, should alert healthcare givers even in the absence of USG or genetic abnormality. These pregnancies require a close follow-up.

CONCLUSIONS

We observed that pregnant women and healthcare givers are usually stressed with the positive screening for SLOS. Our population has a high consanguineous marriage rate compared with other populations. In spite of this high risk for autosomal recessive diseases, we have not observed any SLOS in our series. In addition, with the high number of STS deficiency cases we detected, we wanted to point out the importance of knowing such a common but underestimated condition as a cause of very low uE3 levels. In especially positively-screened patients without USG abnormality, the patients should receive genetic counseling regarding family history of

STS deficiency and SLOS and mothers should be offered testing for STS deficiency in order to avoid unnecessary further tests and stress for the family whether they have a family history or not. We also recommend the addition of risk estimation for STS deficiency in the second-trimester screening tests, in addition to the SLOS risk estimation, to inform the families and health caregivers.

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