Case Report of Clitoral Hypertrophy in 2 Extremely Premature Girls With an Ovarian Cyst

Anne-Laure Nerré, MD, Pierre Bétrémieux, MD, Sylvie Nivot-Adamiak, MD

abstract

Neonatal clitoromegaly is mainly attributed to in utero androgen exposure secondary to congenital adrenal hyperplasia. We report on 2 extremely premature girls with clitoromegaly, increased androgen levels, no salt wasting syndrome, and ovarian cyst. In case 1, the cyst liquid was aspired during ovarian hernia surgery and revealed high androgen levels. After aspiration, serum androgen levels decreased, as did clitoral size. In case 2, an ovarian cyst was seen on pelvic ultrasound. Aspiration was not indicated. The cyst regressed spontaneously on iterative pelvic ultrasounds, and her clitoromegaly decreased. Case 1 demonstrates the ovarian origin of this transient virilization. Cyst formation seems to be linked to the physiologic maturation of the hypothalamic-pituitary-ovarian axis. Thirteen cases of clitoromegaly with hyperandrogenism, without salt wasting syndrome, have been reported in extremely premature infants. In the context of clitoromegaly, we recommend ruling out in utero androgen exposure, adrenal hyperandrogenism, and disorders of sex development. We further recommend affirming hyperandrogenism by androgen assay and confirming ovarian origin with gonadotrophin assays and pelvic ultrasound. Drug therapy abstention and clinical and ultrasound monitoring are recommended because spontaneous regression of clitoral hypertrophy seems to be the most common outcome in the literature, as it was in our 2 observations.

With the increased survival of very premature infants, there are new issues to consider in the identification of clitoromegaly. This anomaly, with specific characteristics of posterior labial fusion and rugosity of the labial skin, is mainly attributed to in utero androgen exposure secondary to congenital adrenal hyperplasia (CAH) in female infants.[1] We present 2 cases of neonatal clitoromegaly associated with ovarian cyst and hyperandrogenism that decreased over time.

METHODS

Clinical and laboratory data were retrospectively collected from the medical records of 2 extremely premature girls with clitoral hypertrophy and ovarian cyst, hospitalized in the NICU of the University Hospital of Rennes, France. Hormonal assay methods at the laboratory of University Hospital are described in Table 1. Assays carried out in case 1 were obtained by radioimmunoassay after extraction and chromatographic separation at the laboratory of the University Hospital of Lyon, France. Consent for the collection of data was obtained from the parents of both children.

CASE PRESENTATIONS

Patient A was born at 24 + 6 weeks' gestation (WG) by vaginal delivery

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Dr Nerré collected the clinical cases and drafted the initial manuscript; Dr Nivot-Adamiak carried out the initial analyses and reviewed and revised the manuscript; Dr Bétrémieux treated the infants and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

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TABLE 1 Hormonal Assay Methods

Assay Method	Manufacturer	Hormones
Radioimmunoassay	Beckman-Coulter, Villempinte, France	DHEAS, aldosterone
Radioimmunoassay	CisBio, Codolet, France	Estradiol
Radioimmunoassay after ether extraction	Beckman-Coulter, Villempinte, France	17OHP, testosterone, androstenedione
Enzymoimmunoassay	Beckman-Coulter, Villempinte, France	AMH
Immunoradiometric assay	CisBio, Codolet, France	ACTH, renin
ADVIA-Centaur Chemiluminescent immunoassay	Healthcare Diagnostics, Saint-Denis, France	Cortisol
Cobas electrochemiluminescene immunoassay	Roche, Meylan, France	FSH, LH

DHEAS, dehydroepiandrosterone sulfate.

in the context of chorioamnionitis. Antenatal management included administration of corticosteroids. Apgar scores were 3 at 1 and 5 minutes. Intubation was necessary at birth. Birth weight was 660 g. There was no family pathology, consanguinity, or drug exposure during pregnancy. She was included in the protocol for the PREMILOC trial study group.[2] Ventilatory support was required for 91 days. Neonatal complications included an episode of acute pulmonary edema that resolved with furosemide, a *Bacteroides ureolyticus* maternal-fetal infection, a catheter line–associated infection, and retinopathy I. Significant fluid and electrolyte disorders were present, requiring large fluid and salt intake, followed by sodium and water restriction. With diuresis, hyperchloremia and hypernatremia resolved, and sodium restriction was necessary. Transient acute kidney injury (AKI) occurred. By day 15, fluid intake decreased, and salt supplementation was stopped. Possible clitoromegaly was reported at birth without any other sign of virilization. At 36 WG, the clitoris measured 15 mm in length and 10 mm in width. The vulva was normal without posterior labial fusion. The anogenital ratio was <0.5. At that time, serum sodium, average blood pressure, and weight gain were normal. Pelvic ultrasound (PUS) confirmed the presence of a uterus and 2 ovaries. The uterus was normal with a uterus/cervix ratio <1. The right ovary was in place and had the expected multifollicular aspect. The left ovary was in the inguinal position with a size of 19 × 8 mm and normal echogenicity with the presence of few follicles. Ovarian vascularization was normal. Karyotype was 46XX. Hormonal assays (Table 2) indicated a low anti-Müllerian hormone (AMH) level, confirming the absence of Sertoli tissue and high androgen levels. A new dosage of steroids was given at 39 + 4 WG by column chromatography (Table 2). 17-hydroxyprogesterone (17OHP) was routinely measured for CAH exclusion, and no therapy was initiated. Clinical ovarian hernia surgery was performed at 41 WG, during which an ovarian cyst measuring 15 × 10 mm was aspired. The withdrawn liquid revealed high androgen levels. One week later, serum androgen levels decreased. By 48 WG, the clitoris size regressed to 12 mm in length and 8 mm in width.

Patient B was born at 27 + 2 WG after a trichorionic triamniotic triplet pregnancy conceived by in vitro fertilization. The mother received 1 dose of antenatal corticosteroids. Birth weight was 960 g, and Apgar scores were 3, 8, and 10 at 3, 5, and 10 minutes of life, respectively. Hyaline membrane disease necessitated surfactant therapy and ventilatory support for 51 days. Neonatal complications included a pulmonary hemorrhage, an AKI (related to fetal distress and ibuprofen treatment), and patent ductus arteriosus. The first days of life were marked by hyponatremia, which corrected quickly, and hyperkalemia, treated with calcium gluconate and furosemide related to transient AKI. The patient had a bifid turgid clitoris that measured 10 mm in length and 15 mm in width. There was no posterior labial fusion with an anogenital ratio <0.5. A uterus was seen on the first PUS, performed at 37 + 5 WG. The right ovary was in the right iliac fossa, measuring 21 × 9 mm with follicles of >5 mm. The left ovary presented a fluid-filled cyst measuring 18 × 23 mm with other small follicles in the periphery. Control at 39 + 1 WG found a left ovary measuring 25 × 26 mm with an increased cyst of 20 × 25 mm. There was no sediment or thickening at the periphery of the cyst. Steroid assays (Table 3) at 38 + 3 WG found high testosterone and androstenedione and nonelevated 17OHP. No hormonal treatment was initiated. By 41 + 3 WG, PUS confirmed spontaneous mild regression of the cyst. The length of the uterus was 27 mm with a uterus/cervix ratio <1. At 41 + 3 WG, the clitoris measured 10 × 10 mm.

DISCUSSION

We report 2 cases of clitoral hypertrophy with serum hyperandrogenism without salt wasting syndrome in 2 extremely premature infants.

Clitoral hypertrophy was probably present at birth. Clitoral size is determined by precise measurements of corpus cavernosum. Normal clitoral width ranges from 2 to 6 mm, and length is <10 mm in term infants.[3] Normal clitoral index (product of the longest sagittal and transverse clitoris) is 15.1 ± 1.4 mm^2.[4] There are few references for premature girls, but in our present cases, measurements were made at term.

TABLE 2 Biochemical Hormonal and PUS Results for Patient A at Corrected Gestational Age

Results	WG						
	34 + 2	35 + 6	36 + 4	37 + 5	39 + 4[a]	41[b]	42
Hormonal assays							
17OHP (nmol/L) <9–term	34.5	16	12.3	23.5	7.4	—	6.1
17-liydroxypregneaolone (nmol/L)	—	—	—	—	22.8	—	—
Progesterone (nmol/L) 0.5–1.3	—	—	—	1.6	—	—	—
11-deoxycortisol (nmol/L) 2.1 ± 1.6	—	—	—	—	2.1	—	—
Testosterone (nmol/L) 0.35–1.24[c]	—	—	6.1	—	3	67.4	3.8
Delta4A (nmol/L) 0.38–3.22[c]	—	—	31.5	—	10.5	196.6	18.1
Estradiol (nmol/L)	—	—	—	—	—	18.9	—
DHEAS (pmol/L) <3.8	—	—	—	5.2	—	—	—
DHEA (nmol/L) 10.5 ± 3	—	—	—	—	38.2	—	—
21-desoxycortisol (pmol/L)	—	—	—	—	<30	—	—
AMH (pmoTL) <75	—	—	8.6	7.5	—	—	—
Corticotropin (pmol/L)	—	—	—	126.9	—	—	—
Cortisol (nmol/L)	—	—	—	7.9	—	—	—
Aldosterone (nmol/L)	—	—	—	5.3	—	1.2	—
Renin (pmol/L)	—	—	—	—	—	0.3	—
PUS/surgery							
Right ovary	—	—	—	—	—	—	—
Left ovary	—	—	Multifollicular ovarian hernia, 19 × 8 mm	—	—	Ovarian cyst	—
Cyst	—	—	No	—	—	Yes, aspiration	—

DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; —, no value.
[a] Radioimmunoassay after extraction and chromatographic separation.
[b] Hormonal assay in the ovarian cyst withdrawn liquid.
[c] Serum values.

TABLE 3 Biochemical Hormonal Results and Pelvic Ultrasound Results for Patient B at Corrected Gestational Age

	WG			
	37 + 5	38 + 3	39 + 1	41 + 3
Hormonal assays				
17OHP (nmol/L) <9 to term	—	7.5	—	4.2
Testosterone (nmol/L) 0.35–1.24	—	2.8	—	1.3
Delta4A (nmol/L) 0.38–3.22	—	9.6	—	5.3
DHEAS (µmol. L) <3.8	—	4.4	—	2.5
Estradiol (pmol/L) <9.9	—	99.1	—	88.1
FSH (mUI/mL)	—	6.9	—	4.6
LH (mUI/mL)	—	4.8	—	3.5
AMH (pmol/L) <74.81	—	—	—	24.9
PUS	First	—	Second	Third
Right ovary	21 × 9 mm	—	15 × 17 mm	23 × 7 mm
Left ovary	Cyst	—	25 × 26 mm cyst	—
Uterus	Present	—	Present	27 mm length
Cyst	18 × 23 mm	—	20 × 25 mm	19 × 20 mm

—, no value.

The absence of a posterior labial fusion (anogenital ratio <0.5) suggests recent virilization.[3]

Further investigations are needed. First, biological hyperandrogenism can be confirmed by hormonal assays (testosterone, androstenedione, dehydroepiandrosterone sulfate). In utero androgen exposure (eg, maternal history, virilization signs during pregnancy) and adrenal origin for hyperandrogenism must be determined. If there are no specific clinical indications of CAH, 21-hydroxylase and 11β-hydroxylase deficiency are routinely ruled out with a normal baseline 17OHP level and a normal 11-deoxycortisol level, respectively. Huysman's study showed significantly higher 17OHP levels and lower cortisol/17OHP in ventilated very premature infants compared with nonventilated preterm infants as well as in more

TABLE 4 Summary Data of the 15 Literature Cases

	Ref 7[a]		Ref 8[b]	Ref 6[c]		Ref 9[d]				Ref 1[e]	Ref 5[f]			Current Study[g]	
	Case 1	Case 2	Case	Patient 1	Patient 2	Patient 1	Patient 2	Patient 3	Patient 4	MS	Patient 1A	Patient 1B	Patient 2	Patient A	Patient B
Year of publication	1990	1990	2003	2004	2004	2008	2008	2008	2008	2009	2012	2012	2012	2017	2017
WG	24	27	25	31	29	26.7	29	25	25	25.7	25	25	24	24.9	27.3
Birth wt (g)	640	1056	490	1556	1400	575	900	635	700	775	550	880	760	660	960
WG[h]	31	35	31.4	36	34	27	29	35	36	36.4	27.1	28	35.4	36.6	38.4
Clitoris size (mm)	—	—	Lg 12	—	—	10 × 4	10 × 4	10 × 4	10 × 4	18 × 13	32[i]	50[i]	50[i]	15 × 10	10 × 15
Karyotype	46XX	46XX	46XX	—	—	46XX	46XX	46XX	46XX	46XX	46XX	—	46XX	46XX	—
PUS	Inguinal hernia	—	Normal	OC	—	OH	—	Normal	—	Normal	Normal	—	—	OH	OC
Ovarian cyst	No	—	No	Yes	—	No	—	No	—	No	No	—	—	Yes	Yes
Salt wasting syndrome	No	No	—	—	—	—	—	—	—	No	No	No	No	No	No
17OHP[h] (nmol/L)	27.2	22	2.5	5	13.8	21.2	14.3	8.6	—	19 3	434	205	128	123	7.5
Testosterone[h] (nmol/L)	0.5	—	7.9	1.8	—	2	2.6	1	1.6	10.8	3.5	3.4	4.4	6.1	2.8
Androstenedione[h] (nmol/L)	—	—	10.4	3.4	—	—	>35	>35	11.8	—	—	—	4.7	31.5	9.6
DHEAS[a] (μmol/L)	6.8	—	9.6′	3.6	9	26.6	6.5′	6.2	5.6	27.1	—	—	—	5.2	4.3
FSH[h] (UI/L)	—	—	—	—	—	91.8	170	41.5	68.5	—	—	—	—	—	6.9
LH[h] (UI/L)	—	—	—	—	—	111.9	162	33.3	86.4	—	—	—	—	—	4.8
CYP21	—	—	—	—	—	—	—	—	—	—	No	No	No	—	—
Treatment	HC, plastic surgery	—	—	No	No	No	No	No	No	No	HC and Florinef	HC and Florinef	HC and Florinef	Asp	No

Asp, aspiration of ovarian cyst; DHEAS, dehydroepiandrosterone sulfate; HC, hydrocortisone; OC, ovarian cyst; OH, ovarian hernia; —, no value.

[a] Reference values for reference 7 not reported.

[b] Reference values for reference 8 not reported.

[c] Reference values for reference 6: 17OHP, <20; testosterone, <0.5; androstenedione, <1; DHEAS, <0.5; FSH and LH, not reported.

[d] Reference values for reference 9: 17OHP, <20; testosterone, <0.3; androstenedione, 0.22–2.4; DHEAS, <10; FSH, <167; LH, <54.

[e] Reference values for reference 1: 17OHP, 3.2–22; testosterone, <0.22–2.8; androstenedione, not reported; DHEAS, 0.14–3.01; FSH and LH not reported.

[f] Reference values for reference 5: 17OHP, <10; testosterone, <1; androstenedione, <2.1; DHEAS, FSH, and LH not reported.

[g] Reference values for current study: 17OHP, <9; testosterone, 0.35–1.2; androstenedione, 0.38–3.2; DHEAS, <3.8; FSH, and LH not reported.

[h] At first investigations.

[i] Clitoral index, normal range =15.1 ± 1.4 mm^2; Lg = length.

severely ill infants. This suggests an insufficient adrenal response to stress in sick, ventilated, very preterm infants. The adrenals increase the production of 17OHP without being able to convert it to cortisol.[5] The elevated 17OHP levels may indicate a relative deficiency of 21- and/or 11β-hydroxylase enzymes, leading to increased androgen production.[6]

In 2004, Greaves et al[7] reported 2 similar cases and concluded that hyperandrogenism was exclusively due to a prolonged activation of the fetal adrenal cortex based on the results of urinary steroid metabolite levels. However, it is difficult to conclude that there was an exclusively adrenal origin because there was no PUS nor gonadotrophin assays performed in their second patient. Moreover, the presence of an ovarian cyst in their first patient was not considered a possible cause of transient hyperandrogenism. Gonadal origin, particularly a disorder of sex development, must be ruled out with a XX karyotype, the absence of testicular tissue (low AMH), and multifollicular ovaries and uterus present on ultrasound. Ultrasound can also reveal an ovarian cyst.

An ovarian cyst was present in both of our cases. In patient A, high androgen levels in the cyst liquid confirmed the ovarian origin of the hyperandrogenism. In patient B, clitoral hypertrophy and the ovarian cyst decreased simultaneously. The absence of a posterior labial fusion suggests later exposure to androgens, which may have come from the ovarian cyst. In the literature reviewed since 1990, we found 13 similar cases, but the link with an ovarian cyst was never reported. Only 1 ovarian cyst was found on 7 PUS performed in these 13 patients. Table 4 provides a summary of these data.[1,6–10]

The pituitary gonadal axis is active during the last trimester of pregnancy. At birth, levels of gonadotrophins rise rapidly between 2 and 4 months of age in response to the postnatal fall of estrogens. This latency is attributed to immaturity of the hypothalamic-pituitary-ovarian axis. The axis then becomes sensitive to the negative feedback of low levels of sex steroids, resulting in the fall of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to prepubertal levels.[11]

Fetal follicles and ovarian cyst formation are stimulated by fetal gonadotrophins and maternal hormones.[12] Because of a shortened gestation, the expected third trimester dips in gonadotrophins levels do not occur, allowing gonadotropin-releasing hormone, FSH, and LH to remain at high levels and stimulate the ovarian follicles,[13] which may lead to ovarian cyst formation and hyperandrogenism.

Greaves et al[10] in 2008 described 4 similar cases. The clitoromegaly was associated with high ex utero levels of LH and androgens for a few weeks. The LH levels were significantly higher compared with other infants of similar gestational age without clitoromegaly.[13] This significant difference in the serum levels of pituitary hormones for extremely preterm infants compared with those for late preterm and full-term infants was confirmed by Greaves et al[14] in 2015. The authors assumed that this higher LH/FSH ratio caused the biological hyperandrogenism and therefore the transient virilization. Androgens were probably produced by the ovaries, but only 2 of 4 patients had a PUS, and no ovarian cyst was found. An adrenal origin was excluded.

Patient A demonstrates that this transient hyperandrogenism had an ovarian origin. Unfortunately, gonadotrophins were not analyzed in patient A. This cyst formation seems to be linked to the physiologic maturation of the hypothalamic-pituitary-ovarian axis in these extremely premature girls.

CONCLUSIONS

In cases of clitoromegaly, we recommend ruling out in utero androgen exposure, adrenal hyperandrogenism (17OHP, 11-deoxycortisol), and disorders of sex development (karyotype, AMH, and PUS). We also recommend affirming hyperandrogenism with hormonal assays (testosterone, androstenedione, dehydroepiandrosterone sulfate) and confirming ovarian origin with gonadotrophin assays (LH, FSH) and PUS to determine if there is an ovarian cyst. Cysts should not be aspirated as a routine procedure in these patients because they often regress spontaneously. If aspiration is required, testosterone and androstenedione should be assayed from cyst fluid. Drug therapy abstention and clinical and ultrasound monitoring are recommended because spontaneous regression of clitoral hypertrophy and ovarian cysts seems to be the most common outcome in these observations.

ABBREVIATIONS

17OHP: 17-hydroxyprogesterone
AKI: acute kidney injury
AMH: anti-Müllerian hormone
CAH: congenital adrenal hyperplasia
FSH: follicle-stimulating hormone
LH: luteinizing hormone
PUS: pelvic ultrasound
WG: weeks' gestation

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