Congenital bilateral Anorchia:  
A Study of 5 Cases in Jordan  
Haidar Bustanji, Nahla Khawaja, Kamel Ajlouni1*

Abstract

We report the clinical and hormonal findings in 5 cases of bilateral anorchism. Five male patients aged 3-5 years presented with suspected cryptorchidism. Physical examination, hormonal, imaging, chromosomal, and molecular analyses of these cases were performed. Ultrasonography of the pelvis and magnetic resonance of the abdomen were performed and failed to show any true testicular tissue or showed only atrophied suspicious testicular tissue. Chromosomal analysis revealed a normal male karyotype and molecular analysis did not reveal mutations or polymorphisms in the SRY gene. The basal FSH and LH levels were increased, and there were increase in response to gonadotropin-releasing hormone test, testosterone levels failed to increase after hCG administration. Lastly, surgical exploration confirmed the absence of testicular structure in three of them. Diagnostically, the very low anti Mullerian hormone level combined with the lack of testosterone response to hCG are the hormonal hallmarks of bilateral congenital anorchia.

Keywords: Cryptorchidism, Testis, Anorchia, Testicular Vanishing Syndrome.

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Introduction

Bilateral non-palpable testes in infancy and childhood may be a manifestation of bilateral cryptorchidism, anorchism, abnormal sexual differentiation, or retractile testicles. Searching for testicular tissue is very important for future patient management(1). Anorchia is the absence of testes in a 46 XY individual with a male phenotype(2). The familial occurrence of the disease and the association of this phenotype with 46 XY gonadal dysgenesis have led to the suggestion that genetic factors may be involved(3). Alternatively, exploratory laparoscopy suggests that anorchia may be caused by a prenatal testicular vascular accident associated with torsion during testicular descent(4).

Testes are determined by the presence of the SRY gene located on the Y chromosome. Mutations involving the SRY gene are associated with a failure of testes determination manifested by the 46 XY gonadal dysgenesis. However, several studies have failed to identify mutations in this gene in patients presenting with anorchia(5). Currently, a conventional (3 to 5 days) hCG stimulation test, with the measurement of testosterone levels before and after stimulation, is widely

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used to determine the presence of hormonally functional testicular tissue. Furthermore, in boys with nonpalpable gonads, only those with testicular tissue should have detectable serum concentrations of anti Mullerian hormone. A very low or undetectable level of serum anti Mullerian hormone in prepubertal boys is more sensitive than measurement of serum testosterone alone for the identification of children with vanishing testes(6).

We screened a cohort of 5 boys with bilateral anorchia with a conventional hCG stimulation test and we measure the serum anti Mullerian hormone levels (all prepubertal). The objective of this study was, therefore, to analyze the clinical and hormonal findings in five prepubertal cases of bilateral anorchism.

Case Reports

Case 1

A 10-year-old boy who presented at our Endocrine Clinic at the age of five years for the evaluation of short stature, microphallus, and absence of both testicles. He was a product of a full-term vaginal delivery. Bilateral undescended testicles had been diagnosed at birth, the scrotum was small and empty, the penis was small with urethral opening at the tip and findings in the rest of the physical examination were unremarkable. The family history was free of urogenital disorders, and the parents were unrelated. The right testicle was atrophic and high in the groin as reported by an expert ultrasonographer, while the left testicle was not detected.

No testicular tissue could be detected by magnetic resonance imaging.

On the first visit, the patient’s height was 104 cm (5th percentile), and he weighed 19 kg. No dysmorphic features were observed, and the scrotal sac was empty and underdeveloped. No palpable masses were found in the inguinoscrotal area. Stretched penile length was 4.5cm. The rest of the physical examination was unremarkable. The karyotype was 46 XY and the sex-determining region of the Y chromosome (SRY) was positive. The bone age according to the Greulich and Pyle method was 4 years and 3 months. Liver, kidney, and thyroid function tests were normal. Basal serum luteinizing hormone (LH) was 0.4 IU/L (prepubertal normal range, 1 to 6 IU/L) and follicle stimulating hormone (FSH) was 8.1 IU/L (prepubertal normal range, 1 to 6 IU/L). After the administration of luteinizing hormone releasing hormone (LHRH) (100 µg, intravascular), the level of LH reached 8.125 IU/L and the level of FSH reached 30.5 IU/L (Table 1). A prolonged hCG stimulation test was done by giving 1500 IU hCG injections twice weekly for 3 weeks. Plasma samples for testosterone before and after hCG administration did not differ (0.05 nmol/L vs. 0.06 nmol/L). Serum anti Mullerian hormone concentration was 0.07 ng per milliliter (normal value for 8 year boy 2.3-139 ng per milliliter)(7).

No surgical exploration was done because the parent refused, but repeated pelvic ultrasound and pelvic MRI at the age of 7 years failed to detect any testicular tissue.

The patient had regular follow up and at the current visit, he is 10 years old with a height of 124 cm (–2 standard deviation) and a weight of 24.5 kg (10th percentile). The growth velocity over 5 years was 4 cm per year. Stretched penile length was 6 cm (mean stretched penile length for his age is 6.4 ± 1.1
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Findings in the rest of the physical examination were unremarkable. Evaluation showed a bone age of 8 years.

Case 2
A 10.5-year-old boy presented at our Endocrine Clinic at the age of 6 years for the evaluation of bilateral absent testes. Bilateral undescended testes had been diagnosed at birth. The karyotype showed a normal male karyotype 46 XY with positive SRY. At the age of 6 years, the basal serum testosterone level was 0.07 nmol/L, compared to 0.08 nmol/L after daily intramuscular administration of 1500 IU hCG for 3 days. On examination, the patient had a hypoplastic scrotum with no palpable testes. Stretched penile length was 6 cm with the remaining physical examination unremarkable. An expert ultrasonographer did not detect any testicular tissue in the abdomen, pelvis, or inguinal areas. The radiological bone age was 5 years and 8 months. At the age of 8 years, his basal FSH was 19.9 IU/L (prepubertal normal range, 1 to 6 IU/L) and LH of < 0.5 IU/ml (prepubertal normal range, 1 to 6 IU/L). After administration of 100 µg LHRH intravascular, FSH reached 61.2 IU/L and LH reached 7.3 IU/L. A prolonged hCG stimulation test was done by giving 1500 IU hCG injections twice weekly for 3 weeks. Plasma samples for testosterone before and after hCG were unchanged at 0.07 nmol/L. Serum anti Mullerian hormone concentration was 0.09 ng per milliliter. At the current visit, he is 136 cm tall (25th percentile) and weighs 41 kg (95th percentile). The growth velocity over 4 years was 5 cm per year.

Case 3
A 7.5-year-old boy was referred to our Endocrine Clinic when he was 5.5 years old for the evaluation of absence of both testicles and micropenis. A review of medical records revealed that the patient was a product of a full-term vaginal delivery, part of a twin pregnancy (his brother has no medical problem). Bilateral undescended testes had been diagnosed at birth. Pelvic ultrasound could not detect the left testicle in either the scrotum or the inguinal pelvic regions, but there was an oval-shaped structure in the right inguinal canal, which was suspected to be the right testicle. He received hCG therapy (1500 IU intramuscularly twice weekly for 6 weeks) with no change in the position of the right inguinal oval-shaped structure. The karyotype was 46 XY and SRY positive. Laparoscopic surgery was performed at the age of 2 years, and no testicular tissue was found in the abdominal cavity. At the age of 4 years, the pelvic MRI showed no testicular tissue in the inguinal areas or the abdominal cavity, but a small cystic structure was seen on the right side of the abdomen, which was assumed to be the right testis. To evaluate this finding further, the patient underwent another laparotomy, which failed to detect testicular tissue.

At presentation, he was 5.5 years old, with a height of 116.5 cm (95th percentile), and weight of 21 kg (95th percentile). Findings on the physical examination showed an empty hypoplastic scrotum, stretched penile length of 4 cm, 2 standard deviation (SD) below the mean for his age(6). Bone age was matching. GnRH stimulation tests showed an increment of FSH from 1.62 IU/L to 13.4 IU/L and LH from 0.01 IU/L to 1.4 IU/L. A short hCG stimulation test was performed, and the basal serum testosterone level was 0.01 nmol/L, with the fourth day serum testosterone level reaching 0.03 nmol/L. Serum anti Mullerian hormone concentration was 0.6 ng per
milliliter (normal value for 6-year-old boy 4.9-265 ng per milliliter)\(^7\).

At the current visit, he is 7.5 years old, with a height of 134 cm (above 95\(^{th}\) percentile), weight of 31 kg (95\(^{th}\) percentile) and stretched penile length of 5 cm (mean for this age is 6.2 cm ± 1)\(^8\). (Fig B).

**Case 4**

A 15-year-old boy, with both testicles absent, had regular follow-ups at our Endocrine Clinic for the last 5 years. A review of medical records revealed that the patient was a product of a full-term vaginal delivery.

Bilateral undescended testicles had been diagnosed at the age of 4 months. Pelvic ultrasound tests could not detect testicles in either the scrotum or the inguinal pelvic regions. Abdominal computerized tomography (CT) scans did not detect any testicular tissue. The buccal smear was negative for Barr bodies. The karyotype was 46 XY and SRY positive. At the age of 1 year, he underwent exploratory surgery for testicular tissue detection, and only flimsy vas deferens and vessels were found ending in a small atrophic testis. The test is descended to the scrotum but did not show any enlargement in the following months. Another exploration was done shortly thereafter by a pediatric surgeon, and no testicular tissue could be detected. Laparoscopic surgery was performed at the age of 6 years with no testicular tissue or vas deferens seen on both sides.

At the age of 10 years, the patient’s height was 136 cm (50\(^{th}\) percentile) and the weight was 31 kg. Besides a scar in the right inguinal area, he had hypoplastic scrotum without wrinkling, and the testes were not palpable. Basal serum testosterone level was 0.03 nmol/L and after a short hCG stimulation test, the serum testosterone levels remained unchanged. Basal FSH was 3.7 IU/L, LH was < 0.07 IU/L, and after 100 µg intravenous administration of LHRH, FSH peaked to 54 IU/L and LH peaked to 9 IU/L. Serum anti Mullerian hormone concentration was 0.12 ng per milliliter.

The patient had regular follow up at our clinic. At the age of 13 years, his serum testosterone was 0.08 ng/ml, FSH 124.5 IU/L, and LH 24 IU/L. Induction of puberty with intramuscular testosterone was done at the age of 14 years with some good response. The initial dose was 50 mg/month for the first 6 months, which was increased to 100 mg for another 6 months. The dose will continue to be increased gradually to adult replacement dose\(^9\).

At the current visit, the patient is 15 years old, with a height of 165 cm (25\(^{th}\) percentile) and weight of 51 kg (25\(^{th}\) percentile). Stretched penile length was 12 cm with pubic hair tanner stage (IV).

**Case 5**

A 19-year-old boy is doing regular follow up at our Endocrine Clinic as a case of bilateral testicular absence. He was a product of a full-term vaginal delivery. An empty scrotum was noticed from birth. A pelvic ultrasound procedure could not detect testicles in either the scrotum or the inguinal pelvic regions. Abdominal CT scans did not detect any testicles. The karyotype was 46 XY and SRY positive. Laparoscopy was done at the age of 1 year, and no testicular tissue was found in the inguinal areas. Another operation was done at the age of 5.7 years, and no
testicular tissue was found in the abdominal cavity.

A short hCG stimulation test was performed at the age of 5 years with basal testosterone of 0.06 nmol/L, which remained unchanged on the fourth day.

The patient had regular follow up at our clinic, and the induction of puberty was done at the age of 14 years with some good response by using a monthly intramuscular testosterone regimen\(^9\). At the current visit, he is 19 years old, with height of 171 cm (25\(^{th}\) percentile) and weight of 62 kg (25\(^{th}\) percentile). Stretched penile length was 13 cm with good pubic hair and tanner stage IV. Other findings during the physical examination were unremarkable.

**Discussion**

Bilateral congenital anorchia affects one in 20,000 males and unilateral congenital anorchia affects one in 5,000 males\(^{10}\). Although some patients with anorchia present with ambiguous external genitalia or micropenis, the majority of cases have a normal male phenotype. The male differentiation of the genital tract and the development of the penis and scrotum are dependent on the production of anti-Mullerian hormone (AMH) and androgens. In boys with nonpalpable testicles, only those with testicular tissue should have detectable serum concentrations of AMH\(^{11}\). Lee et al.\(^6\) compared AMH in 17 boys with nonpalpable gonads (age at diagnosis, 2 days to 11 years) serum AMH concentration was 0.7 +/-0.5 ng per milliliter, as compared with 39.5 +/-37.6 ng per milliliter in the 48 children with testes (P<0.001). In another study he defined the normal range for age for AMH concentration, and a measurable value below the lower limits for males is indicative of either dysgenetic testicular tissue or absent gonads\(^{7}\). We measured AMH in four of our patients all the result was below the lower limits for their age (Table 1).

Special attention should be paid to the SRY gene when dealing with a male patient with anorchia. However, in all the 5 cases discussed, there was a positive SRY region, and these results match with other published data\(^{12}\). Mutations such as the Insulin Like 3 (INSL3) gene, which is necessary for correct testicular descent\(^{13}\) and an SF1 mutation (NR5a1), which, is a nuclear receptor transcription factor play a key role in regulating adrenal and gonadal development, especially when micropenis is also present. In a cohort of 24 boys with bilateral anorchia, SF1 mutation was found in one case with normal adrenals and micropenis\(^{14}\). Two of our patients had micropenis associated with bilateral anorchia. However, the SF1 gene mutation was not studied.

Clinical findings need to be confirmed by provocative tests such as GnRH and hCG stimulation tests. In vanishing testicular syndrome, the GnRH test induces a peak and prolonged elevation of FSH and LH levels, as demonstrated in 3 of our patients\(^{15}\). The hCG stimulation test has several protocols with the short one being a 3-day stimulation test using 1500 IU daily. Samples for testosterone analysis are collected before the first injection and 24 hours after the last injection\(^{16}\). The stimulation test may be prolonged for 3 weeks using bi-weekly hCG injections to confirm that the gonads are dysgenetic\(^{17}\). In our patients, the hCG stimulation test via both protocols demonstrated the lack of testosterone response with no change in the serum levels. However,
the hCG short test was more convenient. It is also important to note that when basal FSH and LH are elevated, GnRH and hCG tests are not necessary for the diagnosis of anorchia because increased LH secretion has an effect similar to that of hCG administration on testosterone secretion\(^{(18)}\). Two of our patients had elevated basal FSH indicating primary gonadal failure.

**Table 1. Endocrine evaluation of 5 male patients with anorchia**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr) at presentation</th>
<th>Years Flu-up</th>
<th>Basal +hCG</th>
<th>ultrasound</th>
<th>Testosterone (nmol/liter)</th>
<th>FSH (IU/ml)</th>
<th>LH (IU/ml)</th>
<th>DNA analysis</th>
<th>AMH Level</th>
<th>Age at surgery (yr)</th>
<th>Testes at surgery</th>
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<tr>
<td>1</td>
<td>4.5</td>
<td>5</td>
<td>&lt;0.05</td>
<td>Undetectable</td>
<td>8.1 30.5 0.4 8.1 46 xy positive 0.07</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>7</td>
<td>0.07 0.08</td>
<td>Undetectable</td>
<td>19.99 61.2 &lt;0.5 7.3 46 xy positive 0.09</td>
<td>___</td>
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<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>5</td>
<td>0.00 0.03</td>
<td>Undetectable</td>
<td>___ ___ ___ ___</td>
<td>46 xy positive 0.6 2</td>
<td>R + L no tissue</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>8</td>
<td>0.05 0.08</td>
<td>Undetectable</td>
<td>124 ___ 24 ___</td>
<td>46 xy positive 0.12 7</td>
<td>R + L no tissue</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>14</td>
<td>0.06 0.06</td>
<td>Undetectable</td>
<td>5.0 42 0.6 4.7 46 xy positive NA 2,10</td>
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<td>___</td>
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<td>___</td>
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</tr>
</tbody>
</table>

**Figure 1:** Male patients with anorchia (A) represents case 1 at 5 years old. (B) Represents case 3 at age 7.5 years with a stretch penile length of 5 cm

**Consent**

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.
Ultrasonography is usually useful in confirming the anorchia diagnosis even though it is very difficult to differentiate between enlarged inguinal lymph node and testes. The sensitivity of the procedure also decreases if the testes are situated in the lumbar area. Magnetic resonance imaging may be the most precise method for locating pelvic or abdominal testes, but children usually need sedation for the long scanning time(19). All our patients had ultrasound scanning several times and due to uncertainty, some had exploratory surgeries which revealed no functional testicular tissue.

Pediatric laparoscopy has evolved as an important tool in the search for impalpable testes. If the testicular vessels end blindly, it implies that the ipsilateral testis is absent and no further surgical exploration is necessary(20). Exploratory operations were done to detect the testicular tissue in three of our patients before puberty with procedures repeated several times due to the insistence of the parents, without any change in the original diagnosis.

Linear growth and maturation are influenced by several factors that act in concert to modify an individual’s genetic growth potential. Nutritional status, exercise, growth, and thyroid hormones are the main players until the onset of the pubertal spurt, which occurs due to interactions between the growth hormone (GH) and sex hormones(21). The ability of testosterone to stimulate pituitary GH secretion appears to be transient and is expressed only Prepubertal. GH and Insulin like Growth Factor 1 (IGF1) concentrations decrease significantly during late puberty and into adult hood, despite continued elevated concentrations of gonadal steroid hormones in normal individuals(22). Linear growth was not affected in our group, and two patients had successful puberty induction by gradually increasing doses of intramuscular testosterone injection starting at the age of 14 years(9).

CONCLUSIONS

In boys with the 46 XY karyotype and nonpalpable testes, a systematic effort to locate the testes is necessary. Failed conventional hCG-induced testosterone responses during childhood should be combined with basal gonadotropin levels to evaluate anorchid patients and very low or undetected AMH will validate the diagnosis of Anorchia. Also, with the presence of good ultrasonographic evaluation a pediatric laparoscopic procedure or exploratory laparotomy may be unnecessary.

List of abbreviations

- hCG - human Chorionic Gonadotropin
- XY - sex chromosomes
- SRY - sex-determining gene located on the Y chromosome
- LH - luteinizing hormone
- FSH - follicle stimulating hormone
- GH - growth hormone
- GnRH - gonadotropin releasing hormone
- LHRH - luteinizing hormone releasing hormone
- SF1 mutation (NR5a1) - nuclear receptor transcription factor
- IGF1 - insulin like growth factor 1
- AMH - anti Mullerian hormone
- INSL 3 - insulin like 3
- CT - computed tomography
- MRI - magnetic resonance imaging
References

متلازمة اختفاء الخصيتين: دراسة لخمسة حالات في الأردن

جمال منجي، نهله الخواجة، كامل العجلوني

الملخص

عدم العثور على الخصيتين عند الأطفال الذكور (مظهرًا وحيوينًا 46 XY) قد يكون ناجمًا عن مرغ الخصية الهائرة أو تشوته التطور الجنسي أو في حالات نادرة عن متلازمة اختفاء الخصيتين. ومن الأمراض تشير هذه الحالة عن حالات الخصية الهائرة في التحول البطني التي تتحمل إمكانية التحول السرطاني مع الزمن. نبهر هذا الحالة السريرية والوراثية والدراستية والتحفزيئة خمسة ذكور مع مقارنة النتائج بالعمليات الجراحية التي أجريت لبحث عن الخصيتين حيث نرى أنه يمكن الاعتماد على الفحوص التشخيصية بدون اللمحة للجراحة.

الكلمات الدلالة: الخصيتين، متلازمة اختفاء الخصيتين، التطور الجنسي.