Pituitary–gonadal axis in male under masculinisation

K L Ng, S F Ahmed, I A Hughes

Abstract

Aims—To study the value of assessing serum concentrations of luteinising hormone (LH), follicle stimulating hormone (FSH), testosterone, and dihydrotestosterone (DHT) in patients with male under masculinisation not caused by androgen insensitivity.

Methods—A retrospective study of a register of cases of male under masculinisation (20 with abnormal testes, eight with 5α-reductase deficiency, three with testosterone biosynthetic defects, seven with Drash syndrome, and 210 undiagnosed).

Results—A human chorionic gonadotropin (hCG) stimulation test was performed in 66 of 185 children with male under masculinisation. In 41 of 66 patients the dose of hCG was either 1000 U or 1500 U on three consecutive days. The rise in testosterone was related to basal serum testosterone and was not significantly different between the two groups. Testosterone:DHT ratio in patients with 5α-reductase deficiency was 12.5–72.8. During early infancy, baseline concentrations of LH and FSH were often within normal reference ranges. In patients with abnormal testes, median pre-LHRH (luteinising hormone releasing hormone) concentrations of LH and FSH were 2 and 6.4 U/l, respectively, and post-LHRH concentrations were 21 and 28 U/l. An exaggerated response to LHRH stimulation was observed during mid-childhood in children where the diagnosis was not clear and in all children with abnormal testes.

Conclusions—The testosterone:DHT ratio following hCG stimulation is more reliable than the basal testosterone:DHT ratio in identifying 5α-reductase deficiency. During infancy, the LHRH stimulation test may be more reliable in identifying cases of male under masculinisation due to abnormal testes than basal gonadotrophin concentrations.

Keywords: pseudohermaphroditism; under masculinisation; androgen insensitivity syndrome

Male under masculinisation is a disorder of sexual differentiation characterised by incompletely masculinised external genitalia in an individual with XY karyotype, bilateral testes, and male internal genital tracts. Recognised causes of male under masculinisation include gonadotrophin deficiency or resistance as well as a defect in androgen biosynthesis or action. Diagnostic schemata recommend karyotype, pelvic ultrasound scan, testosterone, and dihydrotestosterone (DHT) concentrations with human chorionic gonadotropin (hCG) stimulation, gonadotrophin concentrations, and androgen receptor binding in genital skin fibroblasts.1 While age related reference ranges for serum gonadotrophins and testosterone are available for healthy children, results of these parameters are rarely available in children with male under masculinisation.1

We have recently reported the results of these investigations in cases of confirmed androgen insensitivity syndrome (AIS).1 However, most cases of male under masculinisation do not have molecular abnormalities in the androgen receptor gene or abnormalities in androgen receptor binding, and therefore remain undiagnosed.1 A study of the biochemical abnormalities in this large group of patients

Table 1

Histology report in cases of male under masculinisation classified with “abnormal testes.”

<table>
<thead>
<tr>
<th>Case</th>
<th>Left testis</th>
<th>Right testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No data available</td>
<td>Absent Leydig cells, dysplastic, fibrosis</td>
</tr>
<tr>
<td>2</td>
<td>Dysplastic, fibrosis</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Absent Leydig cells</td>
<td>Absent Leydig cells</td>
</tr>
<tr>
<td>4</td>
<td>Absent Leydig cells</td>
<td>Absent Leydig cells</td>
</tr>
<tr>
<td>5</td>
<td>Absent Leydig cells, fibrosis</td>
<td>Absent Leydig cells, fibrosis</td>
</tr>
<tr>
<td>6</td>
<td>Immature seminiferous tubules</td>
<td>Immature seminiferous tubules</td>
</tr>
<tr>
<td>7</td>
<td>Absent</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>8</td>
<td>Extensive fibrosis</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>9</td>
<td>Dysplastic</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>10</td>
<td>Dysplastic</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>11</td>
<td>Dysplastic</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>12</td>
<td>Dysplastic</td>
<td>Dysplastic</td>
</tr>
<tr>
<td>13</td>
<td>Dysplastic</td>
<td>Dysplastic</td>
</tr>
</tbody>
</table>

Table 2

Details of cases of abnormal testes and cases where the diagnosis was unknown

<table>
<thead>
<tr>
<th>Abnormal tests</th>
<th>Diagnosis unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 years</td>
<td>1–13 years</td>
</tr>
<tr>
<td>Basal LH, n</td>
<td>9</td>
</tr>
<tr>
<td>Median age</td>
<td>0.1</td>
</tr>
<tr>
<td>Range (0.03–0.5)</td>
<td>(2–8)</td>
</tr>
<tr>
<td>Basal DHT, n</td>
<td>2</td>
</tr>
<tr>
<td>Median age</td>
<td>0.4</td>
</tr>
<tr>
<td>Range (0.3–0.5)</td>
<td>(6.5–8)</td>
</tr>
</tbody>
</table>

Baseline, pre-hCG; Peak, post-hCG; T, testosterone.
may further improve our approach to evaluation of male undermasculinisation. This report relates to information collected on children with male undermasculinisation held on a database in Cambridge. This database was created with the support of a number of general paediatricians and paediatric endocrinologists throughout the UK.

**Patients and methods**

The UK Database of Ambiguous Genitalia and Intersex Disorder was set up to investigate AIS and consisted mainly of cases of male undermasculinisation where other causes had been excluded. The diagnosis was entered as reported by the clinician. Among the 700 entries in the register, there were 395 cases of 46XY male undermasculinisation: eight with 5α-reductase deficiency, three with a defect in testosterone biosynthesis, seven cases of Drash syndrome, 20 cases of abnormal testes, 210 cases of AIS, and 147 cases where the diagnosis was unclear. The diagnosis of abnormal tests was reached on the basis of abnormal histology in 13 of 20 cases (table 1); in the remainder, it was based on inadequate response to hCG stimulation. The diagnosis of 5α-reductase deficiency was confirmed by analysis of urinary steroids or detection of a mutation in the 5α-reductase type II gene. Cases of confirmed AIS (n = 210) have been reported previously and were excluded from this study. Cases where investigations had been performed only after gonadectomy were also excluded.

Data analysed included age at investigation, dose of hCG used in the stimulation test, and serum testosterone (nmol/l), DHT (nmol/l), luteinising hormone (LH) (U/l), and follicle stimulating hormone (FSH) (U/l) concentrations. The results of these tests were available in a variable number of cases (table 2). Hormonal analyses were performed by standard immunoassays in endocrine laboratories participating in a national external quality control service (UKNEQAS). Interassay variability for the assays ranged between 10% and 20% (data courtesy of UKNEQAS). Assay results are presented as medians and 90th centiles (P10, P90); intergroup comparison was performed by the Wilcoxon signed rank test. Testosterone, LH, and FSH values were compared with those described in normal children.

**Results**

**TESTOSTERONE, DHT, AND hCG STIMULATION**

Basal serum testosterone reached peak concentrations at around 2 months of age, declining to low concentrations in childhood and rising again postpuberally. Twenty nine of 50 measurements performed in the first year of life were within the age related reference range (fig 1). Of the 66 cases where an hCG test was performed, 25 (38%) and 16 (24%) cases received hCG 1000 U and 1500 U on three consecutive days, respectively. Thirteen different protocols were used in the remaining 24 cases, ranging from 200 U on three consecutive days to 1500 U on five consecutive days. The median rise in testosterone with 1500 U hCG stimulation was 2.6 times the basal concentrations (P10, P90 = 1, 29.4). The median testosterone increment was 4.2 nmol/l (P10, P90 = 0, 14.3). In comparison, the median rise in testosterone with 1500 U hCG was 6.5 times the basal level (P10, P90 = 1, 28) and the median testosterone increment was 3 nmol/l (P10, P90 = 0, 19.2). There were no significant differences in the results obtained using the two regimens. In the two largest groups of patients (abnormal testes and unknown), the rise in testosterone and testosterone increment were not related to the age at which tests were performed. However, there was a significant relation between these two parameters and the basal concentration of testosterone in the group of patients where the diagnosis was unclear (r = 0.3, p < 0.05). The response to hCG stimulation was most notable in 5α-
reductase deficiency with peak median testosterone of 15 nmol/l (range 3.1–38; fig 2). The basal and stimulated testosterone:DHT ratios were higher in those cases diagnosed as 5α-reductase deficiency (table 3). The highest value in the remainder of the cases was 16.8. There was no clear age related change in testosterone:DHT ratio before or after hCG stimulation (fig 3).

GONADOTROPHINS AND LHRH STIMULATION
Basal LH and FSH were observed to be highest during infancy. Over the first year of life, 10 of 20 LH measurements and 13 of 16 FSH measurements were within the normal reference range. Two of these 10 normal LH values and five of 13 normal FSH values were observed in cases with abnormal testes. In the latter group, the median basal concentrations of LH and FSH were 2 U/l (P10, P90 = 1.1, 7.3) and 6.4 U/l (P10, P90 = 2.6, 29.7), and peak stimulated LH and FSH were 21 U/l (P10, P90 = 4.5, 31.4) and 28 U/l (P10, P90 = 9.7, 79), respectively. An exaggerated gonadotrophin response to LHRH stimulation was observed during mid-childhood in those children where the diagnosis was unclear and in infants as well as children with abnormal testes (table 4).

Discussion
The results of this study indicate that while testosterone and gonadotrophin concentrations are often determined in the undermasculinised male, hCG and LHRH stimulation tests are performed in less than half the cases. Basal testosterone and gonadotrophin concentrations followed the pattern seen in normal children with an early peak at 1 to 2 months of age during infancy followed by low concentrations in childhood. The mechanisms underlying the transient postneonatal surge in testosterone are ill understood. The concurrent rise in gonadotrophin concentrations suggests that it is centrally mediated, which can be abolished in primates with a gonadotropin releasing hormone (GnRH) agonist. The decline in testosterone and LH in childhood is thought to be due to increasingly sensitive negative feedback in the pituitary–gonadal circuit as well as a central neuroinhibitory mechanism on release of GnRH. Our previous observations as well as those reported in this study show that the neonatal surge of testosterone and gonadotrophins is generally preserved in children with male undermasculinisation with or without evidence of androgen insensitivity. This suggests that this phenomenon may not be due to changes in sensitivity of testosterone feedback.

The hCG stimulation test is a valid indicator of the presence of functional testicular tissue. There is minimal spontaneous testosterone secretion in young boys after the first few weeks of life, so that stimulation of Leydig cell activity with gonadotrophins is needed when investigating prepubertal subjects. A two to three-fold rise in testosterone is generally considered to be normal. A positive testosterone response to a single hCG injection when used as a screening test in the evaluation of hypogonadism is conclusive. We have recently shown that the test can be negative in proven cases of AIS. There was considerable variation in the dosage and duration of hCG stimulation documented in our study, ranging from 200 to 5000 U and one to five days, respectively. The lack of a relation between the dose of hCG and the subsequent testosterone response suggests that the unit dose may not be critical. The magnitude of testosterone response appears to be related to basal testosterone concentrations and, possibly, Leydig cell mass.

Basal and post-hCG stimulated DHT measurements were not often performed in this

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Table 4 Basal and peak serum LH and FSH after LHRH stimulation in cases of abnormal testes and in cases where the diagnosis was unknown

<table>
<thead>
<tr>
<th>Abnormal testes</th>
<th>&lt; 1 year</th>
<th>1–13 years</th>
<th>&gt; 13 years</th>
<th>Diagnosis unknown</th>
<th>&lt; 1 year</th>
<th>1–13 years</th>
<th>&gt; 13 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-LH (U/l)</td>
<td>5.8 (1.7–8.3)</td>
<td>1.3 (1–2.2)</td>
<td>–</td>
<td>3.1 (0.2–25.5)</td>
<td>1 (0.1–22.2)</td>
<td>13.7 (0.5–30)</td>
<td></td>
</tr>
<tr>
<td>Post-LH (U/l)</td>
<td>24.4 (4.1–34)</td>
<td>8.1 (6.3–21)</td>
<td>–</td>
<td>4.4 (1.7–28.8)</td>
<td>5.6 (0.5–64)</td>
<td>25 (17–40.7)</td>
<td></td>
</tr>
<tr>
<td>Pre-FSH (U/l)</td>
<td>6.4 (2–34.5)</td>
<td>3.3 (1–19)</td>
<td>–</td>
<td>4.8 (0.5–50)</td>
<td>1.2 (0.1–15.9)</td>
<td>8.5 (0.5–80)</td>
<td></td>
</tr>
<tr>
<td>Post-FSH (U/l)</td>
<td>34.1 (9.1–105)</td>
<td>25 (12–32)</td>
<td>–</td>
<td>7.1 (0.1–32)</td>
<td>5.9 (0.3–22)</td>
<td>8.3 (5.1–32)</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed as median (range).
series of cases. The ratio of testosterone to DHT following hCG stimulation is regarded as a useful test for 5α-reductase deficiency. The typical pattern is a normal or raised concentration of plasma testosterone with decreased DHT and increased testosterone:DHT ratio, particularly after hCG stimulation. However, the finding of a testosterone:DHT ratio of 12.5 following hCG stimulation in one infant with 5α-reductase deficiency suggests that the ratio may not be completely specific. This value is within the testosterone:DHT range of 1.5–17 reported in normal male infants. A prolonged hCG stimulation test may be a better discriminator than the standard test as well as perhaps urinary steroid analysis. Decreased 5α-reductase activity in genital skin may also help in the diagnosis, although the normal range can extend to very low concentrations. Phallic growth in this disorder should typically respond to DHT cream application which may be an indirect indicator of the diagnosis. Direct molecular genetic testing is an alternative approach that is likely to be diagnostic of 5α-reductase deficiency.

The variable patterns of basal LH and FSH with age were similar to those observed in normal children and children with the syndrome of gonadal dysgenesis. In this study some cases were reported as having “abnormal tests” based solely on the results of an hCG stimulation test as well as their clinical phenotype and karyotype. Our analysis of hCG stimulation tests in AIS showed that some proven cases of this syndrome may have a poor testosterone response so that it is unwise to use this solely for the diagnosis of gonadal dysgenesis. The data in the group of children categorised as having abnormal tests did not show severely raised basal gonadotrophin concentrations, even during infancy. However, an exaggerated LH response following LHRH stimulation was observed. Basal and stimulated FSH concentrations were higher than LH, suggesting that FSH concentrations as an indirect measure of Sertoli cell function may be a more useful indicator of testicular dysfunction. Serum inhibin B concentrations may be decreased in clinical conditions associated with impaired Sertoli cell function and have a reciprocal relation with serum FSH concentrations. A recent study of serum anti-Müllerian hormone measurements in a large group of intersex patients has proposed this Sertoli cell product as a useful marker to distinguish gonadal dysgenesis from isolated defects in androgen production or action.

A register based study has the problem of data reporting from multiple and variable sources. Nevertheless, this disadvantage was more than compensated for by analysing data from a large number of cases of male undermasculinisation where a number of appropriate tests had been performed and reported. The results of this study would have been even more meaningful if a greater number of cases had been thoroughly investigated. Hormone assays were performed in a number of laboratories, but each was a participant in a national external quality control scheme.

Key messages

- Male undermasculinisation is a common cause of genital ambiguity
- Investigation of male undermasculinisation requires thorough evaluation using previously published guidelines
- The hypothalamo–pituitary–gonadal axis is intrinsically active during the first month of life and this is an opportune moment to investigate affected children
- The hCG stimulation test is useful for studying defects in testosterone synthesis as well as 5α-reductase deficiency
- The LHRH stimulation test may be more reliable than basal gonadotrophin concentrations at identifying male undermasculinisation due to abnormal testes

Nevertheless, data from this study do help with interpreting the results of investigations performed in cases of male undermasculinisation which are not due to AIS. While a raised testosterone:DHT ratio following hCG stimulation is generally diagnostic, a normal ratio does not necessarily exclude 5α-reductase deficiency. An acute LHRH stimulation test may be useful to evaluate cases of male undermasculinisation presenting in mid-childhood. Most cases of undermasculinisation, however, currently remain undiagnosed.

As our understanding of the pathophysiology of male undermasculinisation improves, the practice of evaluating the pituitary–gonadal axis in the undermasculinised male using uniform protocols needs to become more thorough if the diagnostic yield in this condition is to be improved. We would recommend the protocol described in the paper by Viner et al. These guidelines, however, are not based on any prospective study of the true value of the investigations detailed. Such a study would require the cooperation of general paediatricians and paediatric endocrinologists nationwide. We would also recommend establishing early contact with a paediatric endocrinology centre so that appropriate investigations can be performed and interpreted as early as possible.

We are grateful to the many clinicians throughout the UK who contributed information about their cases to the database. Support for these studies was provided by the Wellcome Trust and the Birth Defects Foundation. The secretarial assistance of Mrs Norma Coggins is gratefully appreciated.


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