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Objective use of testosterone reveals androgen insensitivity in patients with proximal hypospadias

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Abstract *Objective:* We report preoperative testosterone stimulation based on glans width measurements in patients with midshaft and proximal hypospadias, revealing androgen resistance in those with proximal hypospadias.
Methods: Patients had maximum glans width measured preoperatively. Those <14 mm initially received 2 mg/kg testosterone cypionate intramuscularly for two to three doses, with the aim of increasing glans width ≥15 mm. Not all patients achieved targeted growth, and some were subsequently treated with escalating doses of testosterone.
Results: 5/15 midshaft patients had two to three doses of 2 mg/kg testosterone, with all increasing glans width to ≥15 mm. 29/47 proximal patients had testosterone, with 13 (57%) not reaching desired glans width. Six of these and another six patients had escalating doses from 4 to 32 mg/kg testosterone, with 11 then achieving targeted glans width. Relative androgen resistance was found in 19/29 (66%) proximal cases, including all treated patients with perineal hypospadias.
Conclusions: 39/62 (63%) patients met objective criteria for preoperative testosterone stimulation based on glans width <14 mm, which is less than the average normal newborn glans diameter. Evidence of relative androgen resistance was found in 19 (49%), all with proximal hypospadias.
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Introduction

Preoperative androgen stimulation has been reported to increase glans circumference, penile length, and/or improve prepucial vascularization before hypospadias surgery. To our knowledge, dosing recommendations are empiric, with various regimens of intramuscular or topical androgens administered to patients with a subjectively small-appearing penis before repair. Neither objective size indications for preoperative stimulation nor endpoints to stop treatment have been reported. The aim of our study is to report preoperative testosterone treatment based specifically on glans diameter measurements in patients with primary midshaft and proximal hypospadias.

Patients and methods

Patients

Consecutive patients with midshaft and proximal hypospadias (proximal shaft to perineal) with operations from June 2009 to October 2012 were included. Pubertal patients (Tanner 2 and above) were excluded. Standard preoperative assessment of these patients included measurement of widest transverse glans width using a ruler in the outpatient clinic (Fig. 1), as previously described [1]. Glans width in these patients was compared with measurements we made in both normal neonates undergoing elective circumcision and infants with distal hypospadias. At median ages 5 weeks and 9 months, mean glans widths were 14 mm and 15 mm, respectively.

Preoperative testosterone stimulation was used in patients with midshaft and more proximal hypospadias for glans width $<$14 mm, with the goal of enlargement to $\geq$15 mm. Initially, the preoperative regimen was 2—3 monthly injections of 2 mg/kg testosterone cypionate intramuscularly (IM), with surgery scheduled within 6 weeks after the final dose. Intraoperative measurement of glans width was repeated. When failure to reach the target size was noted in some cases following this protocol, treatment changed to initial injection with 2 mg/kg followed by repeat measurement performed in the outpatient clinic in 3—4 weeks. Those with glans size $\geq$ 15 mm had no further stimulation, whereas patients without growth to this extent next received 4 mg/kg injection and repeat measurement in 3—4 weeks, progressing as needed to 8 mg/kg, 16 mg/kg, etc. Surgery was then scheduled within 6 weeks after the final injection. All injections were testosterone cypionate.

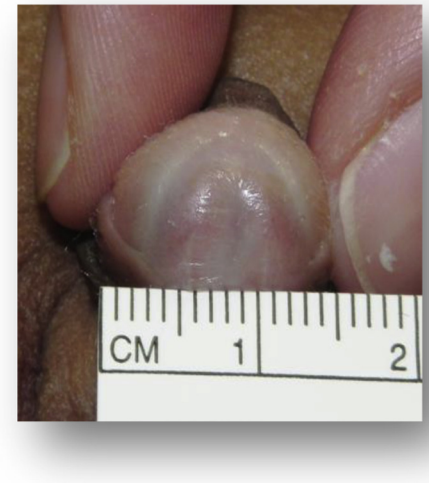

Figure 1 Sample glans size measurement with a ruler in the clinic. The ruler measures the widest point, irrespective of prepuce. Maximum glans diameter in this patient was 14 mm.

Newborns and neonates with hypospadias and glans width $<$14 mm who were initially evaluated in the outpatient clinic at ages $<$6 months had re-examination at 6 months of age to allow for spontaneous growth from postnatal endogenous androgen secretion.

Karyotyping was only done for patients having both hypospadias and undescended testis. Hormonal assays were not performed either before or during testosterone therapy. Syndromic hypospadias was defined as occurring in patients with syndromes known to be associated with hypospadias.

Statistical analysis was performed using Mann Whitney U for between group comparisons. All analyses were performed using Prism 6 for Windows statistical software.

Results

During the study period 62 consecutive patients with primary midshaft (n = 15) and proximal (n = 47) hypospadias were evaluated by WS and NB (Fig. 2). Of these, 28 boys (45%) did not receive preoperative testosterone stimulation. There was no difference in the median age and intraquartile range (IQR) in boys who did versus those who did not receive testosterone, 11 (IQR 8.3—14.5) and 10 (IQR 6—22.2) months, respectively, p = 0.57.

Midshaft patients

Five out of 15 (33%) boys with midshaft hypospadias received preoperative testosterone. Mean glans width in these five patients was 11.6 mm (SD 1.1), versus 15.1 mm (SD 1.4) in the 10 not treated. Two of these 10 boys had outpatient clinic glans measurement $\geq$14 mm, which was found intraoperatively to be $<$ 14 mm when the prepucial hood was retracted, and so by our protocol should have received preoperative testosterone. All five midshaft cases treated with 2 mg/kg testosterone for two to three doses increased their glans width to $\geq$15 mm, to a mean of 16.0 mm (SD 0.8).

Proximal patients

Twenty-nine out of 47 boys (60%) with proximal hypospadias had preoperative testosterone for mean glans width of 11.1 mm (SD 1.7), versus 15.1 mm (SD 1.7) in the 18 not treated. Three of these 18 boys had intraoperative measurements $<$14 mm when the prepucial hood was retracted, and so by the protocol should have received preoperative testosterone.

In the 29 patients with proximal hypospadias who had testosterone stimulation, 23 initially received 2 mg/kg for

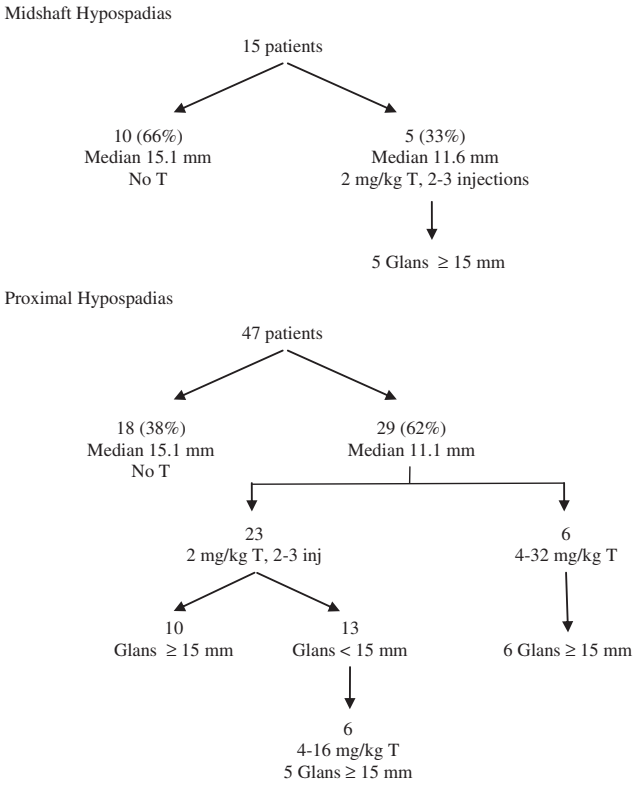

Figure 2 Flow diagram of median glans width in patients with midshaft and proximal hypospadias, separated by those who did and did not receive intramuscular testosterone cypionate at the listed doses.

two to three injections. Of these, 13/23 (57%) did not reach targeted glans width $\geq$15 mm, with no change in four and an increase of 1 mm in four, 2 mm in one, 3 mm in one and 7 mm in one. Mean glans width for these patients after stimulation was 12.8 mm (SD 1.7). Six of these 13, who all underwent 2-stage hypospadias repair with additional stimulation before the second stage, next had escalating doses of testosterone $>$2 mg/kg to achieve glans width $\geq$15 mm. Doses required were 4 mg/kg in four patients, and 8 mg/kg and 16 mg/kg in one each, achieving a glans width of 15—16 mm in all but one case, who received a maximum dose of 4 mg/kg and remained $<$15 mm at surgery.

Another six boys received testosterone on the modified protocol beginning at 2 mg/kg and then progressing to higher doses each month until glans width $\geq$15 mm was achieved. This required 4 mg/kg in three, 8 mg/kg in two and 32 mg/kg in one boy. All reached or exceeded the minimum target, with widths of 15—18 mm, mean 16.5 mm (SD 0.4).

These 29 cases included seven (25%) with perineal hypospadias, all requiring $>$2 mg/kg to reach targeted glans width.

Scant pubic hair was noted in the patient receiving 32 mg/kg. Otherwise no treatment side effects were noted. Bone age in the patient receiving 32 mg/kg 1 year later was normal, but was not determined in other patients.

Karyotyping was done in eight patients for hypospadias and cryptorchidism, as well as an additional patient discovered to have an ovotestis at surgery when tunica vaginalis was harvested to cover the neourethra. Results were normal in seven of nine, whereas one had 45XO,46XY karyotype and another had a chromosome 9 deletion. One patient had Down's syndrome and one had Opitz syndrome. Of these, only the boy with Down's syndrome met criteria for preoperative testosterone, failing therapy with increased glans width from 13 to only 14 mm after two injections of 2 mg/kg testosterone.

Discussion

This is the first report using objective criteria to select patients for preoperative testosterone stimulation. Our interest in glans width began with the observation that glans dehiscence occurred significantly more often following proximal versus distal hypospadias repair despite using the same glansplasty technique and sutures [2]. This prompted routine measurements of glans width in subsequent patients with hypospadias and in newborn controls undergoing elective circumcision. At median age 1 month, mean glans width in normal neonates was 14.0 mm, versus 14.8 mm and 12.9 mm in boys with distal and proximal hypospadias at median age 9 months, p $<$0.001 [1]. Considering a small glans to be a potentially modifiable risk factor for glans dehiscence, we recommended preoperative testosterone in patients with midshaft and proximal hypospadias whose glans width was less than that of a normal newborn, that is $<$14 mm.

We previously reported subjective testosterone use in patients with proximal hypospadias, with 39% treated based on an impression of a small glans and penis. Glans width measurement in the current series should have resulted in 68% of consecutive patients with proximal and 47% with midshaft hypospadias receiving adjuvant therapy for a glans smaller than found in normal newborns. If there is therapeutic value in preoperative testosterone stimulation to increase glans size, objective measurements such as we performed are needed to accurately identify such patients. However, although we found preoperative measurements accurate in most, 5/28 (18%) with outpatient glans width 14—15 mm were found intraoperatively to have a smaller glans that in each case measured 13 mm after the prepuce was retracted. To avoid discomfort we did not separate the prepucial hood from the glans preoperatively to facilitate measurements, although that could be done.

The endpoint of stimulation in patients with midshaft and proximal hypospadias was $\geq$15 mm, corresponding to the average glans width in patients with distal hypospadias who have a low risk for glans dehiscence. Testosterone (2 mg/kg) given two to three times preoperatively achieved this targeted growth in all five patients with midshaft hypospadias. However, similar treatment in 23 boys with proximal hypospadias was successful in only 10 (43%). Relative androgen resistance was demonstrated in 19/29 (66%) proximal patients selected for testosterone therapy, with doses of 4 mg/kg to as much as 32 mg/kg needed to increase glans size to the average width occurring in patients with distal hypospadias. This included all seven patients with perineal hypospadias treated with testosterone.

Others reporting preoperative androgen use have not used either objective criteria for stimulation or endpoints

of treatment. For example, Luo et al. administered 2 mg/kg intramuscular testosterone enanthate monthly for three doses in 25 patients aged 9–12 months with penile shaft to perineal hypospadias and a penis "significantly smaller than usual" [3]. An increase in glans circumference, from which a mean diameter change of 9–12 mm can be calculated, occurred in these patients. Two (8%) did not respond and 10 (43%) of the remaining 23 were considered to have sufficient growth after only one to two injections, although there was no stated endpoint for this decision. Nerli et al. treated 11 boys with proximal hypospadias and "a small penis" at a mean age of 19 months (16–27) using the same adjuvant therapy, resulting in a mean increase in glans diameter from 9 to 12 mm, with two (18%) not responding [4]. Gearhart and Jeffs reported that two doses of 2 mg/kg testosterone enanthate increased glans width from an average of 12 to 17 mm in 36 boys, 11 with distal, 16 with midshaft and three penoscrotal hypospadias (meatal location in another six undergoing reoperation was not stated) [5]. Selection of these patients for therapy was not described.

In each of these series penile length was also measured before and after therapy, and Nerli et al. stated that increases in length "paralleled" increased glans circumference [4]. Our interest in glans size related to glans dehiscence and so we used measurements from normal neonates and compared those with patients with varying extents of hypospadias to establish parameters for therapy. Similar objective criteria could be developed based on stretched penile length, but have not been reported.

Although the purpose of hormonal stimulation in our patients was specifically to reduce glans dehiscence, we do not yet have sufficient follow-up to determine if treatment was successful. To date, only one study reported urethroplasty outcomes related to preoperative stimulation, in which 75 boys mean age 33 months with primary coronal to penoscrotal hypospadias were randomized to receive 2.5% transdermal dihydrotestosterone applied to the penile shaft and glans daily for 3 months, or no treatment. All then had TIP repair, with urethroplasty complications developing in 1/37 with, versus 9/38 without topical hormones, $p = 0.01$ [6].

We did not perform endocrine testing on these patients before therapy, as there is no established protocol for such evaluations and Feyaerts et al. reported no significant abnormality detected by endocrine testing in 32 consecutive patients with hypospadias [7].

We also did not perform routine genetic testing, especially in boys without other obvious defects, and so may have underestimated the prevalence of syndromic hypospadias cases.

The partial androgen resistance we observed could have resulted from 5 alpha-reductase deficiency or disordered androgen receptor structure or function. Presumably any defect in androgen synthesis was bypassed by the testosterone injections administered. A large study involving 337 patients (77 "severe" cases) and 471 control participants reported no haplotype tagging polymorphisms involving the 5 alpha-reductase II gene associated with hypospadias [8]. Androgen receptor mutations have been found in less than 5% of tested subjects with hypospadias [9,10]. Decreased androgen receptor function related to CAG and GGN polymorphisms have been investigated with conflicting results in hypospadias patients versus control participants [11,12] although the study by Adamovic et al. found a single nucleotide polymorphism correlated with increased risk for severe hypospadias [8,11,12]. Finally, androgen receptor expression was reported to be increased in the prepuce of boys with severe hypospadias versus distal cases or control participants [13]. Our study does not add information regarding these possible underlying etiologies, but the variable finding of androgen resistance based on changes in glans diameter could help further refine assessments of patient cohorts being evaluated.

Bone age to date has only been determined in our single patient who received 32 mg/kg testosterone, with no advanced age 1 year post-stimulation. Only one study obtained bone age determinations before and 12 months after administering 2 mg/kg testosterone for two doses in 40 boys, reporting that none had advanced bone age [14]. Twelve-month post-stimulation bone age was determined in 20/36 patients receiving the same hormonal regimen by Gearhart and Jeffs, who also found no advanced age [5].

Our study did not address whether boys receiving alternative forms of androgen stimulation, for example using testosterone enanthate or propionate, or testosterone or di-hydrotestosterone cream, or beta-hCG, have similar dose responses. Prior reports comparing injection versus topical applications found no significant differences in penile length or glans circumference changes [4,15], but one trial obtaining serum levels during therapy noted 2/13 (15%) boys receiving topical testosterone reached levels above normal (>10 ng/ml) and 2× the mean increase of those treated by injection (5 ng/ml) attributed to "unpredictable absorption" [15]. We used intramuscular injection to ensure delivery of the prescribed dose; had topical preparations been used, the cause for lack of response could not have been distinguished between partial androgen resistance versus failure to apply the medication unless blood sampling to measure serum levels was also done.

Conclusions

Using a maximum glans width cut-off of <14 mm to administer preoperative testosterone stimulation, 68% of consecutive patients with proximal and 47% with midshaft hypospadias met criteria because their glans was smaller than found in normal newborns. With an endpoint for therapy of glans width ≥15 mm, all treated midshaft cases were successfully stimulated using two to three injections of 2 mg/kg testosterone. However, relative androgen resistance requiring >2 mg/kg was demonstrated in 19/29 (66%) proximal cases.

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Conflict of interest

None.

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