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REVIEW ARTICLE

Testosterone and the child (0–12 years) with Klinefelter syndrome (47XXY): a review

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ABSTRACT

Aim: To review the evidence base for providing testosterone therapy in the infant and prepubertal child with Klinefelter syndrome (KS).

Methods: Major databases were searched to identify articles that addressed the role of testosterone in the development of the male foetus with and without KS and that characterized testicular function in infants with KS.

Results: Infants with KS have been shown to have an increased frequency of clinical features consistent with deficient testosterone production. However, there are conflicting results regarding whether testosterone levels are low or normal. No direct data address the outcome of therapy in the prepubertal child.

Conclusion: There is an absence of data that directly address the risks and benefits of testosterone therapy in prepubertal children with KS outside of the entity of microphallus. At this time, there is no other documented benefit for testosterone therapy in these children.

ROLE OF TESTOSTERONE IN SEX DIFFERENTIATION

Male sex differentiation was demonstrated to be sex hormone dependent, by A. Jost over 50 years ago. Subsequently, Phoenix et al., generated the 'organizational-activation hypothesis', emphasizing the importance of sex hormones in the sexual differentiation of the brain based on the demonstration of altered mating behaviour particularly in females exposed to testosterone *in utero* (1). In both cases, testosterone was the more potent hormone in achieving an effect either on genital tissues or on mating behaviour. The interpretation by Phoenix et al. was that prenatal exposure to testosterone had an 'organizing action on the tissues mediating mating behaviour' resulting in permanent alteration of behaviour as adults. Gonadal steroids in adults were 'activating' a programmed response.

Recently, Arnold (2) critiqued the 'organizational-activation hypothesis', suggesting that it be expanded to become the 'foundation for a unified theory of sexual differentiation of all tissues'. He noted that 'nearly all tissues show important sex differences in normal function and disease.' His hypothesis that there are functional differences intrinsic to male (XY) and female (XX) cells based on the genetic differences introduced by the presence of a Y chromosome, by X-inactivation or by the presence of paternal X imprinting in females that is lacking in males resulted. Thus, there is not just a simple direct pathway from genetic sex leading to gonads that produce specific hormones, e.g. testosterone and anti-Mullerian hormone, giving rise to male sex rather than female sex. There is rather a dynamic interplay between genes that confer sex-specific gonad determination and the multitude of genes that vary based on being a part of the intrinsic differences in male and female cells. This interplay is influenced by the hormonal milieu resulting in the determined sex as well as sex-specific variance in risk for disease. Wikstrom et al. recently presented data on boys with Klinefelter syndrome (KS) to support this hypothesis as it applies to timing of puberty. They demonstrated that boys with both X chromosomes from the mother entered puberty on average 1.5 years earlier than boys with one X from the father (3).

When approaching the patient with KS, are the manifestations with respect to disease risk a function of the intrinsic differences in the cells with their extra X-chromosome, or a result of testosterone deficiency? More specifically, what manifestations might we expect to be related to testosterone deficiency and therefore amenable to therapeutic

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intervention with testosterone? For the infant and prepubertal child, is there evidence that testosterone deficiency is actually present? This paper seeks to review and establish the knowledge base that addresses these questions for the infant and child.

TESTOSTERONE'S EFFECT ON REPRODUCTIVE TISSUES *IN UTERO*

During foetal development, germ cells arise at approximately 5- to 6-week gestation migrating from the yolk sac to the urogenital ridge (1). Primary sex cords form around the germ cells at 6- to 7-week gestation. Subsequently, at about 9-week gestation, the mesenchyme surrounding the sex cords develops into interstitial tissue which becomes sex steroid secreting Leydig cells. Testosterone and insulin-like-3 (INSL3) production from these Leydig cells along with anti-Mullerian hormone from the Sertoli cells influence the internal genital ducts to differentiate into the male phenotype. Anti-Mullerian hormone production stimulates regression of the Mullerian ducts, while testosterone provides stimulation for the development of wollfian ducts into vas deferens, seminal vesicles and epididymis (4). Male external genitalia develop in response to androgen stimulation of 5α-reductase with resultant dihydrotestosterone production (5). Insulin-like-3 plays a major role in testicular descent (6). Thus, androgen deficiency in the male is expected to result in clinical features of underdeveloped external genitalia that may include microphallus and undescended testes.

TESTOSTERONE USE IN THE INFANT AND PREPUBERTAL CHILD

Testosterone as medication has been used in infancy and the prepubertal child primarily for the treatment of underdeveloped male sexual characteristics, particularly microphallus (7–9). Testosterone levels themselves are documented to be related to the extent of penile growth and length from birth to age 3 years (10). Furthermore, its absence is documented to be associated with poor scrotal and phallic growth (11) and cryptorchidism (6). Thus, if testosterone deficiency is playing a role in the development of boys with KS, one would expect to see underdevelopment of male genitalia as a clinical manifestation.

WHAT IS THE EVIDENCE THAT KS MALES HAVE A TESTOSTERONE DEFICIT IN INFANCY OR CHILDHOOD?

Descriptive studies of boys with KS consistently document an increased frequency of cryptorchidism ranging from 4.5% to 68.75% of referrals (Table 1) (12–16). Among cryptorchid boys, however, KS represented only 1.3% of cases (6).

In contrast, the frequency of microphallus has been less consistent with only one report (15) documenting 56% of boys with KS having microphallus (Table 2). Ross et al. describe a mean penile length = −0.9SDS in 22 infants 1–23 months of age, but only one of those boys met criteria for microphallus at <−2.0SDS. Beyond infancy, mean phallic size is reported to be −1.6 ± 0.8 SDS by Zeger et al. (17), while Robinson et al. (18) initially reported most prepubertal boys with a phallus less than the mean for age. Subsequently, he reported stretched penile length being greater than the mean particularly for older boys, when measurement technique was changed (19). Ratcliffe noted normal newborn phallus but subsequent poor growth in seven of 19 boys followed longitudinally (20). While a small phallus does seem to be common among boys with KS, true microphallus appears to be rare.

Table 1 Cryptorchidism in Klinefelter syndrome

Author (ref no)	Age group	Frequency
Bastida et al. (13)	0.8–12.6 years with KS	11/16 referrals (68.75%)
Ratcliffe (14)	Infants identified by newborn screening (NWBN)	6.3% vs 0.87% controls
Battin et al. (15)	Prepubertally diagnosed KS	13/23 (56%)
Ross et al. (12)	1–23 months with KS	1/22 (4.5%)
Sasagawa et al. (16)	Prepubertal boys with KS	1/20 (5%)

KS, Klinefelter syndrome.

Table 2 Microphallus in Klinefelter syndrome (KS)

Author (ref no)	Age group	Characteristic	Frequency
Bastida et al. (16)	0.8–12.6 years	Small genitalia	2/16 referrals
Ratcliffe (14)	Infants identified by newborn screening (NWBN)	Small phallus	1/90
Battin et al. (15)	Prepubertally diagnosed KS	Micropenis (10–15 mm)	13/23 (56%)
Ross et al. (12)	1–23 months	Micropenis (≥−2.0 SDS)	1/22 (4.5%)
Robinson et al. (18)	2–16 years of age	Less than Prader mean for age	15/16 (94%)

Hormonal values measured during the infant surge at 2–3 months of age have also been variably interpreted. Lahlou et al. (21) reported low testosterone with normal anti-mullerian hormone and inhibin B in infants, while Aksglaede et al. (22) reported normal values. Review of the data in these two papers demonstrates that the KS patients had similar values of testosterone but the relevant norms differed (Table 3). Differences may be the result of the small numbers of KS patients involved (N = 10 each paper) and the slight variation in age of the two populations leading to individual values skewing the results relative to the norm. Data from Ross et al. (12) provide support for this interpretation with the identification of eight boys between 1.5 and 3 months of age whose testosterone values range from 2 to 447 ng/dL (Table 2) giving a mean between the other two papers but with an extremely wide range. Interpretation is also confounded by the variance in what constitutes the timing of the normal neonatal surge. Forest et al. (23,24), Winter et al. (25) and Tomlinson et al. (26) site 2 months as the age of the peak, while Chada et al. (27) site 3–4 months of age for the peak. Population sample variance around the

Table 3 Testosterone values in neonates with KS during neonatal surge

Author (ref no)	Age KS infants (N)	Value KS infants (as listed in paper)	Value KS converted nmol/L	Value normal reference (as listed)	Value normal reference converted nmol/L	Age normal reference (N)
Lahlou et al. (21)	1–3 m (N = 10)	1.17 ± 0.20 ng/mL (X ± SEM)	4.06 ± 0.69			
		Range = 0.29–1.90 ng/mL	1.02–6.59	0.52–4.79 ng/mL	1.8–16.62	1–3 m (N = 215)
Aksglaede et al. (22)	1.8–3.8 m (N = 10)	5.0 nmol/L Median	5.0			
		2.5th–97.5 percentile = 2.2–11.2 nmol/L	2.2–11.2	0.7–8.3 nmol/L	0.7–8.3	2.0–4.5 m (N = 613)
Ross et al. (12)	1.5–3 m (N = 8)	110.43 ± 46.08 ng/dL* (X ± SEM)	3.83 ± 1.6			
		Range = 2–447 ng/dL	0.07–15.51	120–580 ng/dL	4.16–20.13	Upper and lower limits of neonatal surge

*Calculated from data in paper.
KS, Klinefelter syndrome.

true peak may skew the norms to higher or lower ranges confounding interpretation.

Nevertheless, both Lahlou et al. and Aksglaede et al. demonstrate a surge in testosterone in the neonate with KS during the physiological 'mini-puberty' of the male infant. Aksglaede et al., however, point out subtle differences in the ratio of testosterone to LH as well as between FSH and inhibin B, implying that abnormalities in secretory dynamics may be present even if serum testosterone levels are normal (22). A clear deficiency in testosterone during early infancy, therefore, has not been demonstrated; though, alterations in secretory dynamics are probably present. The general pattern of secretion confirms a neonatal surge with mean values at least in the lower end of the normal range for adults albeit with some individuals having high normal values.

Subsequently in the prepubertal period, testosterone values have generally been described as normal (28,29). More recently, Zeger et al. (17) reported as many as 75% of their cohort of 47 boys having a testosterone value less than the 25 percentile for age and Tanner stage. While on an individual basis, many of these values would be interpreted as normal, on a population basis they are in general below the mean and may thus be suggestive of abnormality. How best to translate this into a therapeutic plan for the individual remains to be demonstrated.

Given this limited evidence that testosterone deficiency is present, should we consider testosterone replacement during the neonatal surge in all children with a diagnosis of Klinefelter syndrome or only those with clinical features of testosterone deficiency such as microphallus?

No data yet address these questions. Furthermore, it is unclear whether this strategy would have any untoward effects. While no adverse effects have been reported in the short-term use of exogenous testosterone for microphallus in the androgen-deficient child (8,9), repeated use of exogenous testosterone has been shown to function as an effective contraceptive in adult men (30,31). In KS, the decline in function and involution of the testes is associated with the rise in testosterone in puberty (32). In contrast, studies in adults using aromatase inhibitors rather than exogenous testosterone with the goal of altering the intratesticular testosterone vs the serum level or altering the testosterone:estradiol ratio have been shown to beneficial in men with oligospermia in terms of sperm production (33) and to result in successful sperm retrieval and subsequent pregnancy using intracytoplasmic sperm injection in patients with Klinefelter (34). This would suggest that the patient with KS may have testes more sensitive to the reproductive effects of exogenous testosterone. Its use should therefore be judicious if reproductive function is of concern.

TESTOSTERONE IN INFANCY AND CHILDHOOD ON OTHER THAN REPRODUCTIVE TISSUES

In the human, the influence of androgens on brain and body growth has been most completely evaluated in infancy and childhood in the condition of congenital adrenal hyperplasia. In this condition, the androgens are endogenous, of adrenal origin, and result in prenatal testosterone levels in females above the level of the normal male infant (35). They have been shown to influence the development of the brain with an increase in spatial relations in females exposed to high prenatal androgen levels (36). Males with the condition demonstrate no differences cognitively compared to controls.

For patients with KS, brain morphological differences including decreased left temporal lobe volume of grey matter have been demonstrated in both adults (37) and adolescents (38) with KS. In adults, Patwardhan was able to show that the lower left temporal lobe volume was present in patients not treated with testosterone during puberty compared to either those treated or controls (37). Those individuals who had not received testosterone during puberty also had decreased verbal fluency consistent with the decreased size of the temporal lobe. But these data reflected treatment during adolescence and young adult life, not infancy and childhood.

Evaluation of cognition and motor development in 50 boys with KS ages 4–17 years was carried out by Ross et al.

(39) Only two boys in the cohort had been treated with testosterone in infancy, although nine other boys had received testosterone in the pubertal age range. No cognitive differences were found between those who had been treated with testosterone versus those who had not. The small number of individuals treated as infants or prepubertal children precludes a definitive statement regarding the effect of testosterone in infancy/childhood on brain development in KS. No study has yet addressed differences in brain morphology as a function of testosterone exposure as an infant. Currently, testosterone use for cognitive or neurological differences in the infant and prepubertal child must be considered purely investigational.

Testosterone deficiency in adult males is associated with alterations in body composition and bone mineral density. Amounts of visceral fat have been shown to vary with serum testosterone levels (40,41), while a decline in testosterone levels results in increased total body fat and a decrease in lean mass. Furthermore, treatment of hypogonadal individuals results in a decline in fat mass, an increase in lean body mass as well as an increase in bone mineral density (42). In nonobese ageing males, Allan et al. demonstrated that testosterone therapy prevented the age-associated increase in visceral fat, without a change in total body fat, but with an increase in lean mass. On the other hand, Woodhouse et al. demonstrated that low-dose testosterone was associated with increases in body fat in subcutaneous, intramuscular and intraabdominal depots in young adult males rendered hypogonadal by GnRH agonist therapy, while higher doses resulted in the loss of subcutaneous and intramuscular fat, but no change in intraabdominal fat. Thus, testosterone's effects on fat mass are somewhat variable depending on depot evaluated and baseline clinical status of the individual; though, it consistently increases lean body mass and bone density.

In KS, Ratcliffe et al. (43) describe a progressive rise in subcutaneous fat as measured by skinfold thickness in 68% of 19 boys followed longitudinally through childhood into adolescence. They noted subsequent fat accumulation was limited by supplementary testosterone therapy. Aksglaede et al. (44) describe 18 boys (4.3–18 years of age) with KS having increased fat mass with normal lean mass. Concern regarding the potential of testosterone deficiency as causative or supplementary testosterone as therapeutic in either late childhood or adolescence has been raised. Little is known regarding testosterone's effects on body composition as a result of neonatal/infant or prepubertal exposure. In fact, the role of the infant surge of testosterone between 2 and 3 months of age remains unclear, although Boas et al. (10) have suggested that its importance is for genital growth. Determining the benefits of treatment in infants and children on anything but genital development has yet to be elucidated.

SUMMARY

Infants and children with Klinefelter syndrome can be shown to have an increased frequency of cryptorchidism and small phallus, physical features commonly associated with testosterone deficiency. However, not all children with this syndrome are so affected. Benefits of treatment with testosterone in this age group other than for microphallus have yet to be demonstrated. It is premature therefore to offer testosterone to the infant and child with KS until clear benefits are better described.

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APPENDIX: DISCUSSION FOLLOWING ILENE FENNOY'S PRESENTATION

Testosterone treatment in infancy and childhood

Anders Juul (Copenhagen, Denmark):

Could you clarify the treatment of the three patients who received testosterone (T) therapy in infancy in your studies. What kind of treatment did they receive, and were the indications microphallus in all cases with documented low serum T?

Ilene Fennoy:

All three patients were treated for microphallus with phallus length ≤2.5 cm, but none had documented low T levels in infancy.

Gary Butler (London, UK):

A randomized control trial in Edinburgh showed that the greatest effect of testosterone therapy administered at the onset of puberty was change in body fat. The treated KS individuals developed a normal male pattern of body fat distribution whereas the untreated controlled retained the female distribution. The T treatment also ablated the development of permanent gynaecomastia.

Ilene Fennoy:

Our study confirmed the negative correlation between T and percent body fat, as expected. An unusual finding was the negative correlation between T levels and bone mineral density (BMD), but we must re-analyse our data to take account of what is known about normal BMD and age especially during the changes from late childhood to puberty.