

In Children with 46, XY DSD; HCG Testing Is Not Always the Best Answer for Testicular Function Assessment

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Research Article

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Abstract

Hormonal levels are the hallmark for the assessment of testicular function in XY DSD (Disorders of sex development). Traditionally, it has relied on testosterone level increment after hCG (human chorionic gonadotropin) stimulation testing. More recently role of Sertoli cell hormones is more emphasized.

Objectives: Evaluating the role of serum anti-mullerian hormone and inhibin B on function of the pre-pubertal testis without the need for hCG stimulation test.

Method: The study was conducted in the Endocrinology Clinic in Alexandria University Children's Hospital. All patients who present with XY DSD were tested for testosterone (T), dihydrotestosterone (DHT), Follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-mullerian hormone (AMH), inhibin B. All cases had hCG stimulation test.

Results: The hCG stimulation test was done for 32 cases. There was significant positive correlation between serum testosterone levels before and after hCG stimulation test ($p < 0.001$). Similarly, significant correlation was identified between basal AMH and testosterone increment after hCG stimulation ($p < 0.001$) and between basal levels of AMH and inhibin ($^{MC}p = 0.025$).

Conclusion: Single measurement of basal AMH and/or inhibin B can detect the presence and function of testes by a reliable non-invasive way. Basal AMH assessment is an important tool to distinguish between cryptorchidism and anorchia. hCG test is needed in the work-up of patients with inconclusive results.

Summary

What is known

- Hormonal levels are the hallmark for the assessment of testicular function in XY DSD
- Traditionally, it has relied on testosterone level increment after hCG (human chorionic gonadotropin) stimulation testing

What is new

- Sertoli cell hormones are more appreciated.
- Basal AMH and/or inhibin B can detect the presence and function of testes by a reliable non-invasive way

Introduction

The classification of 46, XY DSD includes defects in testicular development, testosterone biosynthetic defects or its action defect. [1-4]

Traditionally, the standard endocrinological evaluation of 46, XY DSD cases is based on measurement of testosterone, dihydrotestosterone and androstenedione and their ratios either in mini-puberty or after human chorionic gonadotropin (hCG) stimulation which reflects the activity of the testicular Leydig cells. Although it leads to etiological diagnosis of XY DSD cases such as testosterone biosynthetic defects, 5-alpha reductase deficiency or abnormal androgen receptor activity, however, it is cumbersome and needs multiple sampling.[5] More recently, there has been growing evidence of the value of Sertoli cell function assessment as it secretes hormones like anti-mullerian hormone (AMH) and inhibin B. Mullerian ducts have completely disappeared in the male 10 weeks after conception, but testes continue to churn out high amounts of AMH throughout childhood, when basal testosterone and gonadotropin levels have little clinical use. This makes AMH an appealing biomarker for pediatric endocrinologists, not to mention that prior gonadotropin stimulation is not required.[6]

Moreover, it can be used in the evaluation of boys with non-palpable gonads. [7] Inhibin B is another useful marker of normal testicular tissue; therefore, basal inhibin B measurement can be used as a reliable tool to assess both the existence and function of the testes and spermatogenesis afterwards. [8-10]

However, the value of AMH and inhibin B in evaluating cases with XY DSD has been challenged. [2,11] We report our experience of the role of serum AMH and inhibin B in assessing the function of the pre-pubertal testis without the need for hCG stimulation test.

Methods

All cases of 46, XY DSD who were referred to Endocrinology Clinic in Alexandria University Children's Hospital, Egypt for evaluation of atypical genitalia during the period from 1/1/2017-31/12/2019 were included in the study. After informed consent and explanation of the study objectives, peripheral blood was collected for karyotyping, basal serum testosterone (T), dihydrotestosterone (DHT), follicle stimulating hormone (FSH), luteinizing hormone (LH), antimullarian hormone (AMH) and inhibin B.[12] All patients had a Human Chorionic Gonadotropin stimulation test using human chorionic gonadotropin (1500 IU/day) intra-muscular injections for three consecutive days. Venous samples were taken before the test and 24 hours after the third injection, then T/DHT ratio was calculated. We considered functioning testicular tissue when testosterone increment was more than twice the baseline value or absolute testosterone concentration above the upper limit of normal pre-pubertal range. [1,13] Patients with T/ DHT ratio >25 were diagnosed as 5 alpha reductase deficiency and below this value were diagnosed as androgen insensitivity syndrome.[14] Infants in the period of mini-puberty, testosterone and dihydrotestosterone were measured without stimulation and the T/ DHT ratio was calculated. The results of serum hormones were compared with normal reference ranges according to age.[15]

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). [16] The research has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee of the

College of Medicine, Alexandria University. Written informed consents were obtained from all patients' legal guardians

Results

Forty cases were identified with XY DSD. Their age of presentation ranged between zero day to 13 years with a mean of 2.75 ± 3.45

Basal levels of FSH were within normal reference range for age and sex in 35 cases (87.5%) and higher than reference range in 5. Serum LH levels were within reference range for age in all cases. Basal AMH levels were within normal reference range for age in 29 cases (72.5%), 9 cases had low level of AMH and only 2 had high basal AMH. For basal inhibin B, 16 cases (40%) had normal serum level for age, 15 cases had high levels (37.5%), and 9 cases showed low level (22.5%). (Fig. 1)

The hCG stimulation test was done for 32 cases (8 cases were in mini-puberty period that does not need hCG testing). The relationship between serum testosterone level before and after hCG stimulation test is shown in table 1.

After hCG stimulation test, adequate testosterone response (increment) was observed in 26 cases (81.2%). While, the remaining 6 cases had inadequate response. The testosterone/ DHT ratio was calculated in each case. That ratio was exceeding 25 in 14 cases who were diagnosed as 5 alpha reductase deficiency. Furthermore, such ratio couldn't be assessed in one case.

Table 2 shows the correlation between basal inhibin B, AMH, FSH and testosterone response after hCG stimulation test.

Similarly, there was a significant correlation between basal AMH and testosterone increment after hCG stimulation i.e. delta change of serum testosterone ($p < 0.001$). (Fig. 2).

A positive correlation was found between basal level of AMH and inhibin ($^{MC}p = 0.003$). (Table 3)

Discussion

In the current study, both AMH and inhibin B levels were generally lower than normal reference ranges for age in cases with primary gonadal failure, and they were normal in partial androgen insensitivity syndrome (PAIS), and 5 α -reductase deficiency. AMH was higher than reference ranges in 2 cases with androgen insensitivity syndrome while, Inhibin B was higher in 15 cases. Despite these variations, the results showed a positive correlation between mean basal levels of AMH and inhibin B which suggest a discriminatory value of the determination of AMH and inhibin B levels in the diagnosis of XY DSD cases.

Several previous reports [1, 2, 10, 17] have shown that AMH and inhibin B are low in dysgenetic testes, undetectable in patients with anorchia and normal or high in other causes of XY DSD (androgen biosynthesis or action defect, AIS, 5 α -reductase deficiency and Leydig cell hypoplasia). A study in

Denmark showed that the median serum levels of inhibin B hormone in patients with vanishing testes was markedly lower than those in cases of bilateral cryptorchidism. [18]

A significant correlation between AMH and inhibin B was demonstrated in older patients 1-13-year-old in former studies, however, they were not correlated for 0.5-1-year-old patients with XY DSD [2, 19, 20]

Our study found a strong correlation between basal AMH, inhibin B, and FSH levels and hCG induced testosterone increment ($p < 0.001$).

It worth mentioning that the need for hCG stimulation test in the work-up is controversial. Adding to that, there is an extensive range of regimens for hCG stimulation test performance and the definition of an adequate testosterone response to hCG stimulation is also unclear and may depend on the regimen and the age of the child. [15] Our data showed that a low AMH correlates well with a low hCG-stimulated level in most of the cases, however a normal AMH might not predict a normal HCG-stimulated testosterone value as in the cases with LCH. Similar findings were reported by Ahmed SF et al [1] while other investigators found that assessment of serum AMH confirms the testicular function without the need for hCG stimulation tests. [17] In our study, 32 cases -beyond the period of mini-puberty- had hCG stimulation test. Post stimulation testosterone level showed increment after stimulation in most of them (81.2%). There was a positive relation between serum testosterone level before and after hCG stimulation test indicating an adequate Leydig cell function. Patients who did not show adequate post stimulation increment of testosterone (Leydig cell dysfunction) were 2 cases with studied testicular regression syndrome (TRS), 2 with gonadal dysgenesis, 1 with Leydig cell hypoplasia and 1 with 5 alpha reductase deficiency. Post stimulation inadequate testosterone response with lower level of AMH and inhibin B in the case with 5 alpha reductase deficiency is attributed to associated cryptorchidism.

The studied testicular regression syndrome (TRS) cases had very low basal levels of AMH and inhibin B. Stoppa-Vaucher S et al [21] concluded that undetectable AMH level and the absence of Müllerian structures on pelvic ultrasound strongly suggests a diagnosis of bilateral anorchia as early as 3 days of age. Weintraub A et al [22] reported that assessment of AMH can distinguish between cryptorchidism and anorchia. We can conclude that a single measurement of basal AMH and/or inhibin B is highly confirmative of the presence and function of testes. Therefore, basal AMH and inhibin B might substitute the need for

Serum FSH levels, were within normal reference ranges for age in all cases except 3 cases with TRS and 2 patients with gonadal dysgenesis. These cases had high FSH levels with low AMH and inhibin B levels. Assessment of basal FSH levels in patients with XY DSD might elucidate their etiology. High FSH with a low AMH and inhibin B can discriminate cases of defective testicular development from other causes of XY DSD. Moreover, the inverse relationship between circulating FSH and inhibin B levels observed in the current study adds to the current evidence that pre-pubertal Sertoli cells in humans can inhibit FSH release. [18, 23, 24]

On the contrary, basal LH levels were within the normal reference ranges in all cases. Cases with primary gonadal failure showed normal LH levels despite high serum FSH levels. Previous report of 395 cases of under-masculinized 46XY males suggests that FSH is a more useful indicator of testicular dysfunction in the pre-pubertal age group being an indirect measure of Sertoli cell function.

The limitations of this study include a single center experience and a small number of patients.

In conclusion, our study confirmed that a single measurement of basal AMH and/or inhibin B can detect the presence and function of testes. Basal AMH assessment is an important tool to distinguish between cryptorchidism and anorchia. Basal FSH level is an indirect indicator of the Sertoli cell function. Future prospective studies on larger number of patients may decide on the real need for hCG testing in the work-up of these patients.

Abbreviations

AMH: Antimullerian hormone

DHT: dihydrotestosterone

DSD: Disorders of sex development

FSH: Follicle stimulating hormone

hCG: human chorionic gonadotropin

T: testosterone

LH: luteinizing hormone

LCH: Leydig cell hypoplasia

PAIS: partial androgen insensitivity syndrome

TRS: testicular regression syndrome

Declarations

-Conflicts of interest statement:

The Authors declare no conflict of interest.

-Funding sources:

No funding was received for this work from any source

-Code availability:

N/A.

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

-Author's contributions:

- All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission of the final version of the manuscript.

-Study conception and design: Shaymaa Raafat

-Data collection, analysis and interpretation of results, draft manuscript preparation: Shaimaa Raafat, Doaa Khater

-Statement of Ethics:

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the College of Medicine, Alexandria University. (IRB MED# 24/2018).

-Consent to participate:

Written informed consent was obtained from the parents/ legal guardian of participant prior to the study

-Consent for publication:

All authors approve the publication

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Tables

Table (1)

Comparison between serum testosterone level before and after hCG stimulation (n=32)

Testosterone level (ng/ml)	Before	After	Delta testosterone #
Min. – Max.	0.01–3.30	0.05–9.72	0.0–7.78
Mean ± SD.	0.50 ± 0.82	4.03 ± 2.28	3.72 ± 1.97
Median	0.10	4.93	2.77
P	< 0.001*		
#: delta (after – before)			
p: p value for Wilcoxon signed ranks test for comparing between before and after stimulation.			
*: Statistically significant at $p \leq 0.05$			
SD = standard deviation			
hCG = human chorionic gonadotropin			

Table (2)

Relation between testosterone response after hCG stimulation with basal FSH, inhibin B and AMH (n = 32)

	Response of testosterone				Test of sig.	P
	No Response (n = 6)		Response (n = 26)			
	No.	%	No.	%		
FSH						
Low	0	0.0	0	0.0	$\chi^2 = 16.246^*$	FE _p = 0.005*
Normal	2	33.3	26	100.0		
High	4	66.7	0	0.0		
Inhibin B						
Low	5	83.3	4	15.4	$\chi^2 = 6.789^*$	MC _p = 0.003*
Normal	1	16.7	11	42.3		
High	0	0.0	11	42.3		
AMH						
Low	6	100.0	3	11.6	$\chi^2 = 13.812^*$	MC _p = 0.001*
Normal	0	0.0	22	84.6		
High	0	0.0	1	3.8		
χ^2 , p: χ^2 and p values for Chi square test						
MC: Monte Carlo for Chi square test						
FE: Fisher Exact for Chi square test						
*: Statistically significant at $p \leq 0.05$						
FSH = Follicle stimulating hormone						
LH = luteinizing hormone						
AMH = anti-mullerian hormone						

Table (3)

Relation between serum levels of Inhibin B and AMH

AMH	Inhibin B						Test of sig.	P
	Low (n= 9)		Normal (n= 16)		High (n= 15)			
	No.	%	No.	%	No.	%		
Low	6	66.7	2	12.5	1	7.1	$\chi^2=$ 12.180*	MCp= 0.003*
Normal	3	33.3	14	87.5	12	78.6		
High	0	0.0	0	0.0	2	14.3		
Min. – Max.	0.01 – 163.1		17.73 - 171.10		30.70 - 333.0		H= 6.886	0.074
Mean \pm SD.	60.33 \pm 51.61		90.65 \pm 62.18		145.04 \pm 98.96			
Median	48.20		76.80		122.0			
Sig. between stages	p ₁ =0.312, p ₂ =0.003* p ₃ =0.170							
r_s(p)	0.513*(0.004*)							
χ AMH = anti-mullerian hormone								
² , p: χ^2 and p values for Chi square test								
MC: Monte Carlo for Chi square test								
H,p: H and p values for Kruskal Wallis test, Significance between groups was done using Mann Whitney test								
r _s : Spearman coefficient								
*: Statistically significant at p \leq 0.05								

Figures

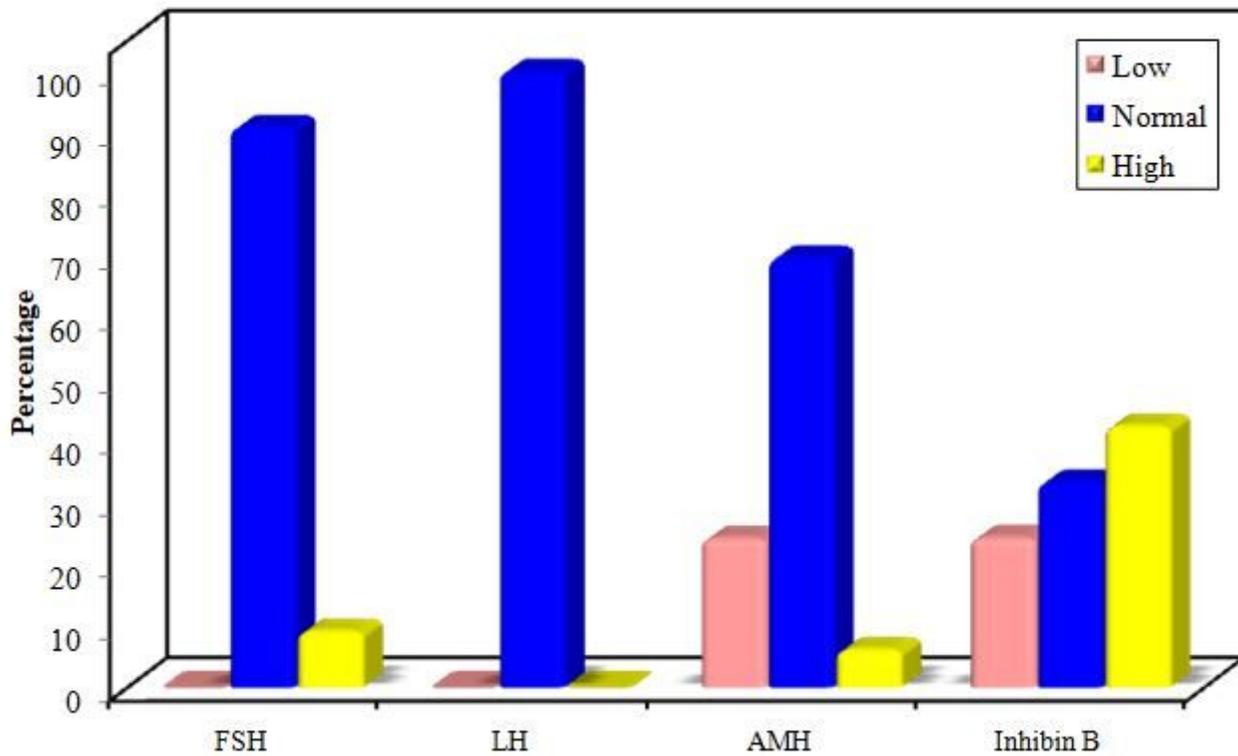


Figure 1

Distribution of the studied cases according to basal FSH, LH, AMH and inhibin B levels for age (n =40)
 FSH= Follicle stimulating hormone LH= Luteinizing hormone AMH= Anti-mullerian hormone

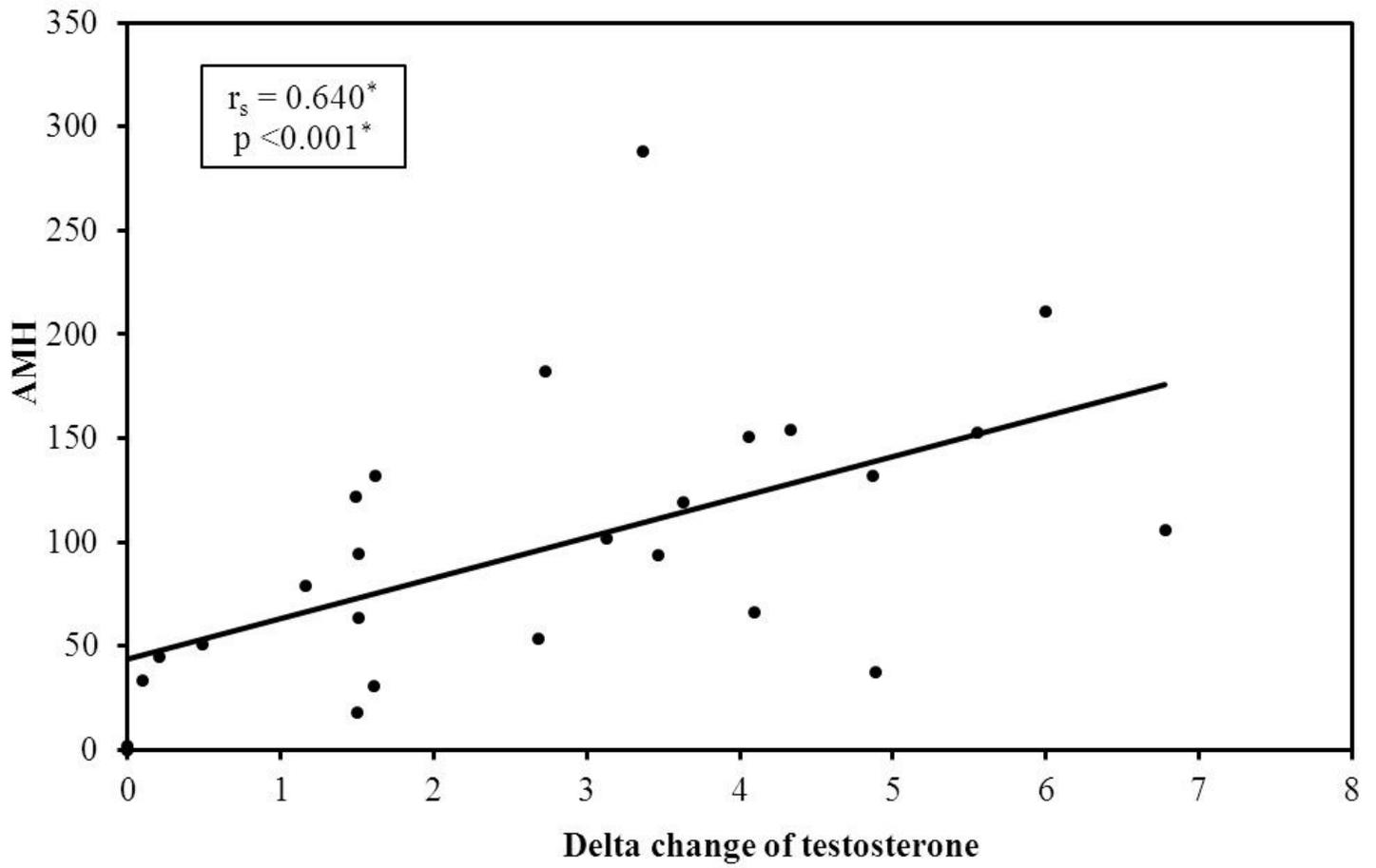


Figure 2

Correlation between Delta change of testosterone and serum AMH. AMH= Anti-mullerian hormone