



ORIGINAL ARTICLE

Value of Anti-Müllerian Hormone in Diagnosis and Management of Children with Disorders of Sex Development

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ABSTRACT

Background: The aim of the study was to investigate the serum levels of Anti-Müllerian hormone (AMH) in children with disorders of sex development and its correlation with other diagnostic investigations to assess the ability of AMH to predict the presence and function of testicular tissue. **Methods:** The study was carried out at the pediatric, pediatric surgery and clinical pathology departments at Zagazig University Hospitals (ZUH) during the period from February 2018 to December 2018. The study was performed on 40 infants and children below the age of ten years. They were divided into two groups, control and DSD groups, each group contained 20 participants. Children were subjected to full history taking, precise clinical examination, and investigations that included ultrasonography, karyotyping and biopsies. In addition, serum AMH, testosterone and dihydrotestosterone (DHT). Statistical analysis using suitable significance tests was performed. **Results:** Specificity and sensitivity of testosterone and DHT in detection of testicular activity were 100% for both while they were 91.7% and 100% respectively for AMH in 46,XY DSD. **Conclusion:** Serum testosterone and DHT had a better diagnostic role in 46,XY DSD than AMH while in 46,XX DSD, AMH is better.

Keywords: AMH, DSD, testosterone, dihydrotestosterone.

INTRODUCTION

Disorders of sex development (DSD) are congenital conditions associated with atypical sex development. They include a wide range of disorders and syndromes that can be classified according to the chromosomal pattern into sex chromosome DSD (Turner syndrome, Klinefelter syndrome, etc.), 46,XY DSD (gonadal dysgenesis, androgen insensitivity syndrome, etc.) and 46,XX DSD (CAH, POR gene defect, etc.) [1]. The global incidence of DSD worldwide is 1/4500 birth [2]. In North, South America and Western Europe, CAH is the most common type, accounting for

approximately 70% of DSD cases [3].

Anti-Müllerian hormone (AMH) is a dimeric peptide hormone secreted by Sertoli cells. It is expressed in male embryos by the seventh gestational week to guide the differentiation of embryo into male genital tract and prevent the involution of Müllerian ducts into female genital tracts [4]. AMH, being a product of Sertoli cells, can reflect the presence and function of testes in boys without any need for testicular stimulation by human chorionic gonadotropin (HCG) hormone [5].

Study design

The study is a case control study that was carried out at Zagazig University Hospitals

(ZUH) during the period from February 2018 to December 2018. It was performed on 40 infants and children who were divided into two groups, control and DSD groups.

The DSD group included 20 members who had a confirmed diagnosis with any type of DSD, while the control group included 20 members who attended to ZUH with minor irrelevant symptoms.

Ethical approval for the study was obtained from the Institutional Review Board (IRB) at the faculty of medicine, Zagazig University. Written informed consent was obtained from all participants' parents. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methods

After history taking, general and local examination the following investigations were done:

- i. **Ultrasonography** was done using high resolution B-mode system with broadband linear probe of 13 MHz.
- ii. **Chromosomal analysis** was done in the molecular and cytogenetics laboratory in ZUH.
- iii. **Gonadal Biopsies:** for 4 patients with bilateral cryptorchidism were subjected to histopathological gonadal biopsies prior to the start of the study.
- iv. **Estimation of serum levels of AMH, testosterone and dihydrotestosterone** by enzyme-linked immunosorbent assay (ELISA)
- v. **Statistically analysis:** was performed using IBM SPSS Statistics, version 24 (IBM; Armonk, New York, USA). Continuous data were presented as Mean \pm SD if normally distributed or median (range) if not normally distributed. Categorical data were presented by the frequency and percentage. Normality was checked by Shapiro-Wilk test. Homogeneity of variance was checked by Levene's test. Chi-squared test of association, Fisher's Exact Test, Kruskal-Wallis H test, unpaired t-test, Mann-Whitney U test and Spearman's correlation were used to detect

significance. Receiver operating characteristic (ROC) curve was used also to measure the accuracy of AMH, testosterone and DHT as diagnostic tests. P -value $<.05$ indicates a significant difference, $P<.01$ indicates a highly significant difference, $P<.001$ indicates a very highly significant difference while, $P\geq.05$ indicates a non-significant difference [6].

RESULTS

Parenteral consanguinity where a highly statistically significant difference between healthy and DSD children was found ($P<.001$), as consanguinity was significantly high in DSD children as shown in Table 1. Children with perineal hypospadias or persistent Müllerian duct syndrome had also either unilateral or bilateral undescended testes shown in Table 2. Five cases of the 6 DSD females included in study were on proper hormonal therapy while only one was presented for the first time during a crisis. Healthy male children had very highly significantly higher anti-Müllerian hormone, higher testosterone and DHT levels than male DSD children ($P<.001$). On the contrary, their levels were not significantly different between healthy female and female DSD children ($P>.05$), as shown in Table 4. Anti-Müllerian f hormone, DHT and testosterone levels were not significantly different in male DSD children grouped according to ultrasonography findings ($P>.05$), as shown in Table 5. Table 6 shows a very highly statistically significant positive strong correlation between serum anti-Müllerian hormone level and testosterone level ($P<.001$).

Table 1.Baseline characteristics of healthy and DSD children

Characteristics	Controls		DSD		Test of significance	P-value
	46X X	46XY	46XX	46XY		
	n=8	n=12	n=6	n=14		
Age (years) Median(range)			1.5(1-4.5)	3(2.5-4.5)	Kruskal-Wallis H test=2.8	.43
Consanguinity, n(%)					Fisher's Exact test=18.4	<.001
Negative	6(75)	8(67)	0(0)	4(29)		
Positive	2(25)	4(33)	6(100)	10(71)		
Age of presentation, n(%)					Fisher's Exact test=5.0	.063
At birth	-----	-----	4(67)	10(71)		
Three months	-----	-----	0(0)	3(21)		
One to 1.5 years	-----	-----	2(33)	1(8)		

DSD, disorders of sex development,

Table 2. Clinical findings of DSD children

Clinical findings	DSD	
	46XX	46XY
	n=6	n=14
Main presentation, n(%)		
Absent both testes	0(0)	8(57)
Absent one testis	0(0)	4(29)
Dehydration and electrolytes disturbance	2(33)	0(0)
Genital ambiguity	4(67)	0(0)
Perineal hypospadias and unilateral absent testis	0(0)	2(14)
Diagnosis, n(%)		
Congenital adrenal hyperplasia (CAH)	6(100)	0(0)
Isolated bilateral cryptorchidism	0(0)	4(29)
Partial gonadal dysgenesis	0(0)	2(14)
Perineal hypospadias	0(0)	4(29)
Persistent Müllerian duct syndrome	0(0)	4(29)

DSD, disorders of sex development, CAH, Congenital adrenal hyperplasia

Table 3. Investigations (ultrasonography and biopsy) in DSD children

US findings	DSD	
	46XX	46XY
	n=6	n=14
Ultrasound, n(%)		
Unilateral undescended testis	0(0)	2(14)
Unilateral undescended testis with female genital organ(s)	0(0)	4(29)
Bilateral undescended testis	0(0)	6(43)
Bilateral undescended testis with female genital organ(s)	0(0)	2(14)
Enlarged adrenal glands	6(100)	0(0)
*Gonadal biopsy, n(%)		
Bilateral Primitive testes	----	2
Unilateral primitive testis with absent testicular tissue on the other testis.	----	2

Table 4. Hormonal profile of healthy and DSD children

Laboratory findings	Cases	Controls	P-value	Cases	Controls	P-value
	46XY	46XY		46XX	46XX	
	n=14	n=12		n=6	n=8	
Anti-Müllerian hormone (pmol/L)	315(25-377)	900(300-1020)	<.001†	19.3±5.9	20±4.4	.81‡
Testosterone (ng/mL)	0.12(0.07-0.15)	0.18(0.16-0.82)	<.001†	0.14± 0.07	0.11±0.03	.28‡
DHT(Pg/mL)	14.8±1.8	27.3±4.3	<.001‡	20.5±5.6	17.8±1.7	.30‡

Table 5. Association between hormonal levels and US findings of male DSD children

Hormones	US findings		Mann Whitney U test	P-value
	Undescended testis and female genital organ	Undescended testis		
	n=6	n=8		
Anti-Müllerian hormone (pmol/L) median(range)	277.5(25-330)	315(248-365)	13.5	.19
Testosterone (ng/mL) median(range)	0.11(0.07-0.13)	0.15(0.1-0.15)	14	.22
DHT(Pg/mL) median(range)	13.2(12.7-16.5)	15.4(12.7-17.6)	13	.18

DHT, dihydro-testosterone,

Table 6. Correlation between anti-Müllerian hormone and testosterone of male DSD children

Testosterone (ng/mL)	Anti-Müllerian hormone (pmol/L)
r	.842
P-value	<.001

r: Pearson correlation, DSD, disorders of sex development. **Total number=14**

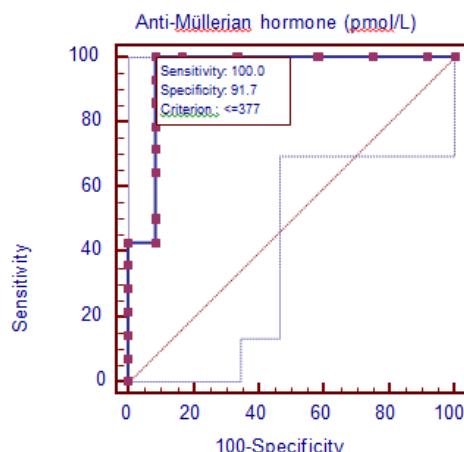


Figure 1. ROC (receiver operating characteristic) curve analysis revealed that area under the curve (AUC) of 0.95 for serum anti-Müllerian hormone (95% CI: 0.858 to 1.047, P<.001). The optimal sensitivity and specificity were (100% and 91.7% respectively at a cutoff expression value ≤ 377).

DISCUSSION

Disorders of sex development can be considered as the most confusing anomalies that may face clinicians. Definite diagnosis of these disorders is necessary for proper gender assignment of patients that may help in proper hormonal or surgical treatment needed for proper sexual development. In addition, proper gender assignment prevents social and psychiatric problems that may face the patients throughout their lives as well as their parents [7].

Anti-Müllerian hormone, (AMH) is produced by Sertoli cells in the seventh gestational week to prevent the development of Müllerian ducts during testicular differentiation. It is also secreted in a tiny amount by the granulosa cells in females to initiate and regulate the recruitment of ovarian follicles. Serum levels of AMH in both sexes vary by age and sex [5].

The present study highlighted the highly significant positive parenteral consanguinity among DSD children in comparison to the healthy children as the positive consanguinity reached 100 % (6/6) in DSD females and 71% (10/14) of DSD males in comparison to 25% (2/8) in healthy females and 33% (4/12) in healthy males. The current results were in agreement with that of Mazen et al. [8]. who reported that about 70% (145/208) of cases had a positive consanguinity. Also, Warne and Raza [9]. stated that almost all DSD cases in India have a positive consanguinity and justified that as most of these disorders are results of autosomal recessive inheritance which requires consanguinity to increase its ability to produce DSD.

In the present study, a major handicap to the study is that the only 46,XX DSD, the study was able to reach, was CAH (6/6)

and this prevented us from investigating the serum levels of AMH in the remaining spectrum of other 46,XX DSD. 67% (4/6) of these CAH females were presented at birth with ambiguous genitalia while the remaining 33% (2/6) were presented latter with dehydration and electrolyte disturbance. The same results were obtained by Mazen et al. [8]. as almost all cases of 46,XX DSD in their study in Egypt were diagnosed with CAH. Moreover, 46,XX testicular DSD is a very rare condition and about 90% of cases are diagnosed at puberty or even after marriage [10]. and this is another limitation to the present study as the age scope of the current study included children only between the first day of life and the age of 10 years.

On the other side, the current study revealed that the most frequent main presentation in DSD males is the absence of both testes which represented 57 % (8/14) of the entire sample. The current study results revealed also that the most frequent types of 46,XY DSD were isolated bilateral cryptorchidism (29%; 4/14), perineal hypospadias (29%; 4/14) and persistent Müllerian duct syndrome (29%; 4/14). The current results disagree with Mota et al. [11]. who performed a retrospective study on 93 patients -known to be 46,XY DSD with ages varying from 4 days up to 28 years old- and stated that the most frequent type of 46,XY DSD was androgen insensitivity syndrome. However, this disagreement may be explained by the different age scopes of the two studies as androgen insensitivity syndrome is mostly presented at puberty when patients- who own external female genitalia and were reared as females- develop primary amenorrhoea according to Hughes et al. [12].

In the present study, there was no significant difference between AMH serum levels in both healthy and DSD females ($P=.81$). Such finding was not surprising as all 46,XX DSD females included in the study were diagnosed with CAH and those patients don't own any testicular tissue as their high titres of androgenic hormones are not products of testicular tissues activity, but accumulation of androgens precursors due inadequate synthesis of adrenal hormones [13]. In addition, 46,XX testicular DSD patients- who are expected to have higher serum titres of AMH- are very rare and rarely diagnosed within the age scope of the current study according to Anik et al. [10].

On the contrary, AMH serum levels showed a very high significant decrease in 46,XY DSD compared with the healthy boys ($P<.001$). The same results were declared by Grinspon and Rey [14], who stated that the serum levels of AMH in boys with anorchia or bilateral undescended testes were unremarkable. In addition, Ahmed et al. [15], performed a cross section study on 284 children in the United Kingdom and found that boys with dysgenetic testes, bilateral undescended testes and anorchia had a much lower serum levels of AMH compared with their healthy fellows. Furthermore, Matuszczak et al. [16], performed a case control study on 50 boys -half of them were cryptorchidic boys and the others were normal- then stated that boys with cryptorchidism (unilateral or bilateral) were found to have a significantly decreased AMH levels compared with the healthy control boys. Also, Hafez et al. [17], reported a significant decrease in serum levels of AMH in boys born with cryptorchidism or anorchia compared with normal healthy males of the same age. However, the previous authors disagreed

with the current study results concerning AMH levels in persistent Müllerian duct syndrome as they reported a nearly normal AMH levels in these patients and explained that as the defect in patients with persistent Müllerian duct syndrome is in AMH-receptors not AMH production. However, persistent Müllerian duct syndrome results not only from a defect in AMH receptors but also its production according to Altincik et al. [18], who stated that persistent Müllerian duct syndrome is caused by a mutation affecting AMH gene or AMH receptor gene type II. In addition, Rane et al. [19], reported that patients with persistent Müllerian duct syndrome have a defect either in production of AMH or its receptors. Furthermore, Kim and Kim [1], mentioned that DSD patient may have more than one disorder at the same time and this might affect the results as all persistent Müllerian duct syndrome patients included in the current study had a unilateral or bilateral undescended testes which certainly can affect AMH production.

Although CAH is characterized by elevated levels of adrenal androgens [20], serum levels of testosterone and DHT in most females diagnosed CAH in the present study showed no significance difference from healthy control fellows ($P=.28, .30$ respectively). Only one female- who was presented for the first time- was found to have higher testosterone and DHT levels. These results were not also surprising as proper hormonal therapy seemed quite successful in reduction the androgenic hormone levels in DSD females utilizing hormonal therapy.

On the other side, serum levels of testosterone and DHT in 46,XY DSD showed a significant drop when compared with healthy boys ($P<.001$). Hafez et al.

[17]. reported that serum levels of testosterone in 46,XY DSD were found to be significantly lower than normal.

In the present study, there was a significantly positive strong correlation between AMH and testosterone levels ($P=.004$).

In the present work, AMH was found to be a good tool in denying existence of functioning testicular tissues in 46,XX DSD patients as its level wasn't affected – compared with control girls- in cases of CAH where testosterone and DHT levels fluctuating by the act of following or not following a regular hormonal therapy. These findings may give a superiority of AMH over androgenic hormones in evaluating the function of testicular tissues in 46,XX DSD patients.

In the present study, the best cut-off value for AMH in discriminating between the existence and absence of functioning testicular tissues was 377 pmol/L. The sensitivity and specificity were 100% and 91.7% respectively. The area under the curve (AUC) reached 0.95 ($P<.001$). Similar results were obtained by Hafez et al. [17]. as they reported that the cut-off value for AMH discriminating between the existence and absence of functioning testicular tissues was 200 pmol/L with a sensitivity of 97% and a specificity of 70%, while the AUC reached 0.90.

Although these findings indicated that serum AMH can be considered as a potential diagnostic biomarker in 46,XY DSD patients below ten years, both testosterone and DHT for the same patients had a much better role as the optimal sensitivity and specificity of them were 100% for both. This finding indicated that AMH has a better diagnostic role in 46,XX DSD than 46,XY DSD patients while on the other side the opposite is present with testosterone and DHT.

The current study revealed that AMH can give rapid reliable data about the testicular existence and function and it can be used as potential diagnostic biomarker in childhood 46,XX DSD below ten years. However, the study revealed also that the cheaper serum testosterone and DHT investigations had a better diagnostic role in 46,XY DSD than AMH.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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