

## Diagnostic accuracy of human chorionic gonadotropins (HCG) stimulation test in XY-disorders of sex development (XY-DSD) presented in Armed Forces Institute of Pathology

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### Abstract

**Objective:** To determine diagnostic accuracy of human chorionic gonadotropins stimulation test in differentiating androgen insensitivity syndrome and 5-alpha reductase deficiency, keeping testosterone to dihydrotestosterone ratio as the gold standard.

**Methods:** The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January to December, 2016, and comprised patients aged 01 day to 20 years having XY chromosomes on karyotyping and with a spectrum of phenotypes. Blood samples were collected from each subject for basal serum testosterone, serum luteinizing hormone and serum follicular stimulating hormone level. Human chorionic gonadotropins stimulation test was performed in every subject as per the protocol. Sandwich chemiluminescence immunoassay technique was used to analyse serum samples. Serum dihydrotestosterone level was also detected to determine testosterone and dihydrotestosterone ratio. Data was analysed using SPSS 24.

**Result:** Of the 104 subjects with a mean age of  $1.78 \pm 0.95$  years, 96 (92.3%) were diagnosed as cases of androgen insensitivity syndrome on the basis of human chorionic gonadotropins stimulation response level, which was 2-9 times of basal serum testosterone level. Also, 8 (7.7%) subjects were diagnosed to have 5-alpha reductase deficiency syndrome. In such subjects, post-human chorionic gonadotropins response level of serum testosterone was more than 10 times of the basal level.

**Conclusion:** The human chorionic gonadotropins stimulation test was found to be comparable to testosterone-to-dihydrotestosterone ratio in differentiating between case of androgen insensitivity syndrome and 5-alpha reductase deficiency.

**Keywords:** HCG stimulation test, Androgen insensitivity syndrome, Disorders of sex development, Dihydrotestosterone. (JPMA 69: 1090; 2019)

### Introduction

Disorders of sex development (DSDs) are congenital conditions that lead to abnormal development of genitalia, both external and internal, due to genetic or chromosomal atypia.<sup>1</sup> In XY sex development disorders (XY-DSDs) there is defect in XY chromosome, resulting either deviation of testicular determination or under-virilisation of geno-typically XY male. This is due to disturbance in either androgen synthesis or androgen action. XY-DSD is a group of disorders that includes androgen insensitivity syndrome (AIS), 5-alpha reductase (5-ARD) deficiency and gonadal dysgenesis.<sup>2</sup> AIS patients can present with complete AIS (CAIS), partial AIS (PAIS) or mild (MAIS). In CAIS, patient has typical female external genitalia, primary amenorrhoea and absent pubic and axillary hair. In MAIS, patient presents with

underdeveloped genitalia, hypospadias, gynecomastia and normal or impaired fertility.<sup>3</sup> About 17.1% babies are born with AIS, which is either diagnosed at birth, or at pre-pubertal or post-pubertal age.<sup>4</sup> Patients with 5-ARD have similar phenotypic appearance. Differentiating AIS from 5-ARD is a real challenge.

Testosterone, for its action in peripheral tissues, has to be converted to dihydrotestosterone by the action of an enzyme called 5-alpha reductase.<sup>5</sup> This is the key enzyme in male sex differentiation. Ideally 5-ARD deficiency should be diagnosed by measuring the enzyme level. Because the facility of measuring this enzyme is not freely available, so testosterone-to-dihydrotestosterone (T-DHT) ratio is calculated for diagnosing 5-ARD deficiency, which is the gold standard for this condition.<sup>6</sup>

Moreover, assay for DHT is also not easily available at most centres and is quite expensive. Alternatively, human chorionic gonadotropins (hCG) stimulation test along with other hormone levels are used to differentiate

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different DSD aetiologies.<sup>7</sup> The current study was planned to document the diagnostic accuracy of hCG stimulation test in differentiating between AIS) and 5-ARD by using hCG) stimulation test while keeping T-DHT ratio as the gold standard.

## Patients and Methods

The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January to December 2016. After obtaining approval from the institutional review board, subjects aged 1 day to 20 years presenting to the facility were recruited. Subjects with XY males on karyotyping and with a spectrum of phenotypes, like hypospadias, azoospermia, gynecomastia and ambiguous genitalia, were included, while all previously-diagnosed cases of AIS and 5-ARD deficiency on treatment were excluded. The subjects were enrolled using non-probability consecutive sampling and informed consent was obtained before collecting blood samples.

On the basis of age, the sample was divided into four groups; group 1 (1 day to 5 years), group 2 (6-10 years), group 3 (11-15 years) and group 4 (16-20 years).

While performing the hCG stimulations test, initial basal value of serum T is measured. Subsequently, 100IU/kg body weight of hCG is given intramuscularly (IM) for three consecutive days and the T level is measured on the 4th day, which is compared with the basal level.<sup>8</sup> In AIS cases, T level after hCG stimulation is 2-9 times of the basal value, while it is more than 10 times the basal level in 5-ARD.<sup>9</sup> About 3.0 ml venous blood was collected in plain gel tube from each subject for basal serum T, serum luteinizing hormone (LH), serum follicular stimulating hormone (FSH), and DHT level. Blood was allowed to clot and serum was separated by centrifugation at 3000g. The hCG) stimulation test was performed in each subject as per laid down protocol.<sup>8</sup> Serum DHT level was also detected to get T-DHT) ratio. Serum LH and FSH analyses were performed on random access assay (ADVIA Centaur® XP Immunoassay System, Siemens) by two-site sandwich immunoassay using direct chemiluminometric techniques. Serum T analysis was also performed on the

same assay by competitive immunoassay using direct chemiluminescent technique. Analysis of serum DHT was done using enzyme-linked immunosorbent assay (ELISA) (BIORAD PhD™ SYSTEM). Data was analyzed using SPSS24. Mean along with standard deviation was calculated for all quantitative variables, like age, serum T, FSH, LH and DHT. Other variables, like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LHRs) were expressed as frequencies and percentages. Receiver Operating Characteristic (ROC) curve was drawn for checking the significance of hCG stimulation test. The level of significance was set at 5%.  $P < 0.05$  was considered significant.

## Results

Of the 154 subjects initially included, 104(67.5%) met the criteria and represented the sample. The mean age of the subjects was  $1.78 \pm 0.95$  years. In terms of age, group 1 had 58(53.8 %) subjects, group 2 had 19(18.3%), group 3 had 25(24.0%) and group 4 had 4(3.8 %) subjects. Overall, 96(92.3%) subjects were diagnosed as having AIS, and 8(7.7%) were diagnosed as 5-ARD-deficient. Parents of 95(91.3%) subjects had consanguineous marriages. With regards to phenotypic presentation, 44(42%) subjects had male phenotype, while 17(16%) had typical female presentation, and 43(41%) had ambiguous genitalia. Also, 27(26%) subjects presented with gynecomastia, 92(88.5%) with micropenis, 71(68%) with undescended testes, and 7(6.7%) presented with hypospadias. Baseline values of all subjects were noted (Table-1).

When it came to the hCG) stimulation test's capacity to differentiate between AIS and 5-ARD cases, its sensitivity was 64%, specificity 71%, NPV 71%, PPV 64%, positive LHR

Table-1: Variables with mean and standard deviation (SD) with significance level.

Variables	Mean $\pm$ SD	p-value
Mean age (years)	1.78 $\pm$ 0.945	
Serum Luteinizing Hormone (LH)(nmol/l)	6.5 $\pm$ 1.24	
Serum Follicle stimulating Hormone (FSH) (nmol/l)	1.7 $\pm$ 1.33	
Serum dihydrotestosterone (pg/ml)	25.17 $\pm$ 25.71	
Mean Testosterone-Dihydrotestosterone ratio	14.72 $\pm$ 4.16	0.000

Table-2: Diagnostic accuracy of human chorionic gonadotropins (hCG) stimulation test.

Test	Sensitivity	Specificity	NPV	PPV	PLHR	NLHR	Accuracy
hCG stimulation test	85.71%	63.03%	93%	40%	2.07	0.42	68.18%

NPV: Negative predictive value

PPV: Positive predictive value

PLHR: Positive likelihood ratio

NLHR: Negative likelihood ratio.

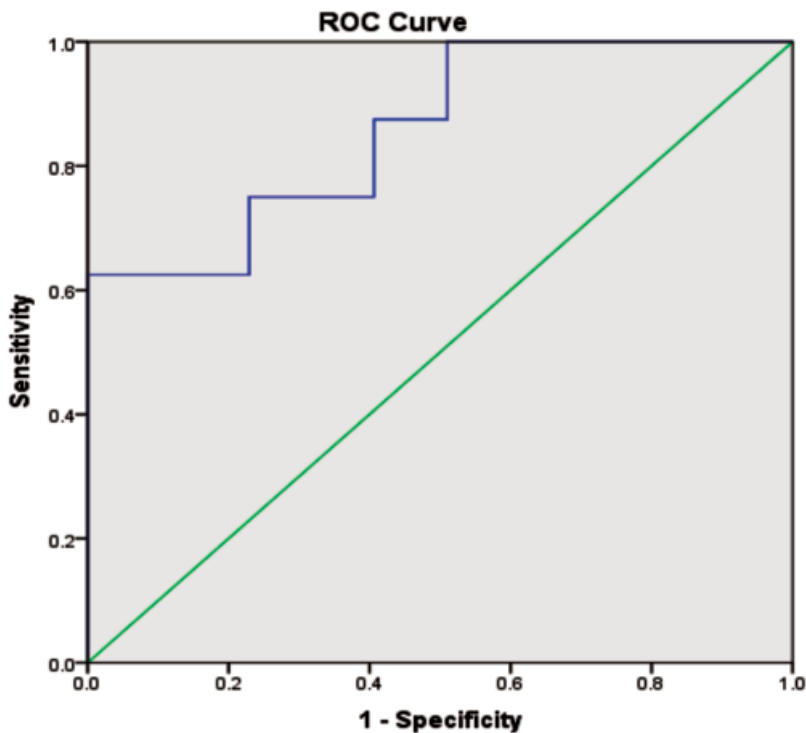


Figure: Receiver operating characteristic (ROC) curve showing validity of human chorionic gonadotropins (hCG) stimulation test.

(PLHR) 2.07, negative LHR (NLHR) 0.42 and accuracy was 68.18% (Table-2).

ROC curve showed area under the curve close to 0.922 (Figure), revealing that this test can easily discriminate between AIS and 5-ARD patients. Comparison of T-DHT) ratio and hCG stimulation test showed significant value ( $p < 0.05$ ).

## Discussion

The study showed that AIS and 5-ARD can be easily differentiated with a simple procedure without resorting to the expansive T-DHT ratio. To the best of our knowledge, there is no such study available from Pakistani population.

AIS and 5-ARD are presently diagnosed with plenty of biochemical and molecular tests which causes a huge financial burden on patients in developing countries like Pakistan. To avoid this, both AIS and 5-ARD can be easily diagnosed by hCG stimulation test. Diagnosis of 5-ARD requires clinical, hormonal and molecular investigations, and is of great importance for appropriate gender assignment and management in general. Variability of phenotypic presentations of AIS with genotype-phenotype variability of identical mutations complicates both the diagnostic procedure and genetic counselling of

the affected families.

The current study showed that hCG stimulation test is 71% specific and 64% sensitive in differentiating the two conditions with 64% PPV. It is 69% accurate with 2.07% PLHE. T-DHT ratio was taken as the gold standard test. Its normal value is below 10. In AIS, its value is between 10.1 and 20, and in 5-ARD syndrome, the value is  $>20$ . Also, the T response after hCG stimulation was taken as the indication test to differentiate between the two conditions. Besides, ROC curve also indicated that hCG stimulation can be reliably used to differentiate between AIS and 5-ARD.

The study corroborates earlier findings. In a study carried out in Oman, all patients with 5-ARD were phenotypically male by birth.<sup>10</sup> But they presented with micropenis, with or without hypospadias. In our study patients of 5-ARD presented with the same clinical features. Most of the patients of 5-ARD have been reported from different parts of world such as Dominican Republic,<sup>11</sup> Turkey,<sup>12</sup> Africa,<sup>13</sup> Guinea<sup>14</sup> and Saudi Arabia.<sup>15</sup>

In another study conducted in Thailand, relation of serum LH, FSH, T, DHT and T-DHT ratio in 5-ARD patients were analysed.<sup>16</sup> Its results matched our results except for LH, which was higher than the reference range while in our study it was within the range.

Protocol of hCG stimulation test followed in most international studies are the same as in our study.<sup>17</sup>

In our study most patients with AIS presented with raised serum LH level. Another interesting feature in AIS patients in our study is that majority of patients had T-DHT ratio between 10.1-20 and response of hCG stimulation was 2-9 times of the basal value. The same results were seen in a study.<sup>18</sup>

Serum LH and product of LH and testosterone-androgen index was raised in AIS patients in our study which is comparable with a study in Brazil.<sup>19</sup>

The current study has some limitations. Due to a lack of patient outcome data, the predictive value of hCG stimulation test versus T-DHT ratio could not be assessed. Moreover, our study sample consisted of individuals who presented to the Endocrine Clinic for investigations of DSDs and, as such, the sample is not truly representative of the population.

A multi-centre prospective study should be undertaken to confirm causality between hCG stimulation test, and AIS and 5-ARD in various ethnic groups of Pakistan. It should be followed by a community-based study to represent the general population.

## Conclusion

Diagnostic accuracy of hCG stimulation test was found to be comparable to T-DHT ratio in differentiating AIS and 5-ARD.

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**Conflict of Interest:** None to declare.

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