TO DETERMINE CUT OFF OF FERTILITY PROFILE OF PATIENTS WITH ANDROGEN INSENSITIVITY SYNDROME (AIS) AT DIFFERENT AGES IN PAKISTANI POPULATIONS

Waqas Hanif, Naveed Asif, Muhammad Younas*, Zujaja Hina Haroon, Qurat Ul Ain, Noreen**

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Combined Military Hospital Malir/National University of Medical Sciences (NUMS) Pakistan, **Combined Military Hospital Multan/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: Current study was aimed to determine cut off value of fertility profile in patients with Androgen Insensitivity Syndrome (AIS) presented at different ages tertiary care hospital of Rawalpindi, Pakistan.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP) Rawalpindi, from Jan 2016 to Dec 2017.
Methodology: Ninety-one (91) patients diagnosed as cases of AIS were included in the study. Subjects were consecutively selected as per inclusion and exclusion criteria. Blood samples were collected from each subject for basal serum testosterone, serum luteinizing hormone (LH) and serum follicular stimulating hormone (FSH) level. Human chorionic gonadotropins (hCG) stimulation test was performed in each subject as per laid down protocol. Sandwich chemiluminescence immunoassay technique was used to analyze serum testosterone, LH and FSH. Serum dihydrotestosterone was also analyzed to calculate testosterone and dihydrotestosterone (T/DHT) ratio.
Results: Mean age of subjects was 1.78 ± 0.95 years. Cut off values of serum LH (IU) according to age in patients with AIS are; 6.80 (1 day to 1 year), 6.74 (1 year to 10 years) 6.6 (10 years to onward), serum FSH (IU) 9.71 (1 day to 1 year), 9.01 (1 year to 10 years), 10.01 (10 years to onward), serum testosterone (ng/dl) 107.32 (1 day to 1 year), 120.76 (1 year to 10 years), 98.32 10 years to onward) before and 310.39 (1 day to 1 year), 354.71 (1 year to 10 years), 293.43 (10 years to onward) after hCG, serum dihydrotestosterone (pg/ml) 22.72 (1 day to 1 year), 26.32 (1 year to 10 years), 21.59 (10 years to onward) and T/DHT 13.65 (1 day to 1 year), 13.99 (1 year to 10 years) and 13.84 (10 years to onward) respectively. Patients were diagnosed having AIS on basis of hCG stimulation response, with serum testosterone 2 to 9 times of basal level.
Conclusion: In this population based study we concluded that, Cut off values of fertility profile of patient of AIS varies according to age. XY karyotype patients presented with ambiguous genitalia are quite helpful in diagnosis AIS.

Keywords: Androgen insensitivity syndrome, Disorders of sex development, Dihydrotestosterone, hCG Stimulation test.

INTRODUCTION

AIS is an X-linked disorder which is due to mutation in androgen receptor gene leading to resistance to androgen receptors[1]. Clinical presentation of AIS varies from complete resistance of and rogen receptor leading to complete AIS, to partial or mild resistance, leading to partial AIS or mild AIS[2]. In complete AIS patient has typical female external genitalia, primary amenorrhea and absent pubic and axillary hair. While in mild AIS, patient presents with underdeveloped genitalia, hypospadias, gynecomastia and normal or impaired fertility[3].

AIS is the most common cause of ambiguous genitalia at birth and feminization or undervirilization at puberty and can be misdiagnosed or confused with other causes of undervirilization, if not investigated properly[4]. AIS is diagnosed by gene analysis but biochemical markers like serum LH, FSH, testosterone, DHT and T/DHT ratio can also be used for diagnosis of AIS[5]. Although

Correspondence: Dr Waqas Hanif, Department of Pathology, Combined Military Hospital, Multan Pakistan
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reference interval of these biochemical markers according to age are available and these biochemical markers are analyzed at time of presentation, but there is some uncertainty regarding the values of these tests[6].

Patients with AIS at post pubertal age, with testes in scrotum, have increased concentration of serum LH and normal to raised serum FSH concentration, and raised concentration of testosterone relative to healthy male[7]. While patients with pre-pubertal AIS have normal range of serum LH and testosterone.

hCG stimulation test has been performed to check the testicular functioning tissue and to find out testicular biosynthetic `abnormalities[8]. While performing the test, initially basal value of serum testosterone, serum LH and serum FSH are measured. 100 IU/kg body weight of hCG is given intramuscularly for three consecutive days and responded testosterone level is measured at 4th day, which is compared with basal level[9]. In AIS, testosterone level after hCG stimulation will be 2-9 times of basal value[10].

In our study, we have analyzed mean values of testosterone, FSH and LH with reference range of fertility profile of patients with AIS. This analysis should improve the interpretation of investigations performed in patients presented with ambiguous genitalia with XY karyotyping. There is no such study available from our population. So, the results of this study will generate useful database which will be helpful for researchers to plan more studies in future.

METHODOLOGY

This cross-sectional study was conducted at department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, from January 2016 to December 2017 after approval of Institution Review Board (IRB). 104 patients of different ages with XY karyotype were presented at endocrine clinic. hCG stimulation test was performed on these patients. Out of this 104, ninety-one subjects were diagnosed as patients of AIS on the basis of hCG stimulation test. Sample size was 90 which was calculated by using formula:

$$n=z^2 (spec) (1-spec)/d^2 (1-prev)$$[6]

(Where z=1.96, prevalence=17.1% (prevalence of AIS) 10 d=5% at 95% confidence level), spec = specificity = 94.1% (22), sensitivity = 100%.

Patients with AIS were considered as one group but were divided into three subgroups according to age: infants, less than 1 year old; children, 1–10 years old; postpubertal, more than 10 year sold. Data analyzed included age at investigation, hCG stimulation test, serum testosterone (ng/dl), dihydrotestosterone (DHT; pg/ml), LH (U/l), and FSH (U/l).

Subjects with XY males on karyotyping and with spectrum of phenotypes like hypospadias, azoospermia, gynecomastia and ambiguous genitalia were included in study. Subjects with gonadectomy were excluded.

Blood samples were collected from selected subjects by nonprobability consecutive sampling technique after taking the informed consent. About 3.0 ml venous blood was collected in plain gel tube from each subject for basal serum testosterone, serum LH, serum FSH and DHT. Blood was allowed to clot and serum was separated by centrifugation at 3000g. (hCG) stimulation test was performed in each subject as per protocol. Serum was also analyzed for DHT to calculate T/DHT. Serum LH and FSH analysis was performed on random access ADVIA Centaur® XP Immunoassay System, by Siemens with principle two-site sandwich immunoassay using direct chemiluminometric technique. Serum testosterone analysis was also performed on random access ADVIA Centaur® XP Immunoassay System by competitive immunoassay using direct chemiluminescent technique. Analysis of serum DHT was done by BIORAD PhD™ SYSTEM - ELISA (enzyme linked immunosorbent assay). Data was analyzed using statistical package for social sciences (SPSS) version 21. Mean ± range was calculated for all the quantitative variables like serum testosterone, FSH, LH and DHT. Test of normality, Kolmogorove Smirnov test, was

applied which revealing data isparametric. Level of significance was set at 5% with 95% confidence interval. A *p*-value ≤0.05 was considered significant at this level of significance. Assay results were represented in range as 3rd and 97th centiles. Testosterone, LH, and FSH values were compared with those described in normal children in the literature.

RESULTS

Ninety one patients, who fulfilled the inclusion criteria, were included in the study. Mean age of subjects was 1.78 ± 0.95 years. On the basis of age, our study population was divided into three groups; group 1 (one day to one year), group 2 (1 to 10 years), group 3 (10 years onward). These groups included 52 (57.1%), 18 (19.8%) and 21 (23.1%) subjects respectively. Regarding ethnicity, Punjabi were 22 (24.2%), pathan 19 (20.1%), kashmiri 18 (19.8%), sindhi 7 (7.7%), balochi 9 (9.9%), urdu speaking 15 (16.5%) and ethnicity group 1 (1.1%).

Gonadotrophins

Basal serum LH and FSH of ninety-one patients with AIS are shown in fig-1, which shows age related changes in LH and FSH level. Serum LH and FSH are high in subjects during initial first few months of life. During childhood, values of serum LH and FSH remained constant, until increasing again in late childhood. Six out of fifty two serum LH and twenty three out of fifty two serum FSH, performed in first year of life, are higher than reference interval and forty six out of fifty two serum LH and twenty nine out of fifty two serum FSH are within reference range. Two out of eighteen serum LH and eight serum FSH levels are higher than reference range in patient from 1 to 10 years age and thirteen out of twenty-one have high serum FSH than reference interval in patients of age 10 years to onward.

Testosterone and hCG Stimulation

Testosterone concentration in serum of ninety one patients are shown in fig-3. Testosterone concentration of patients with AIS are measured according to ages before and after hCG stimulation test. Testosterone concentration increases initially in first few months of life and then decreases. At near puberty testosterone concentration level increases again in healthy individuals. hCG stimulation test were performed on ninety one patients presented at endocrine clinic AFIP. Fig-2 shows different level of

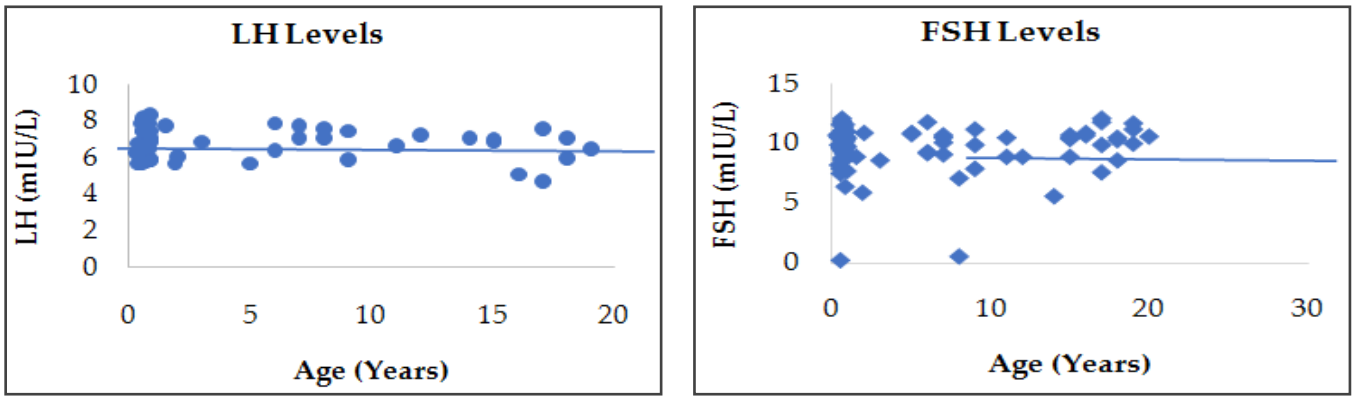

Figure-1: Different values of serum LH and FSH according to ages.

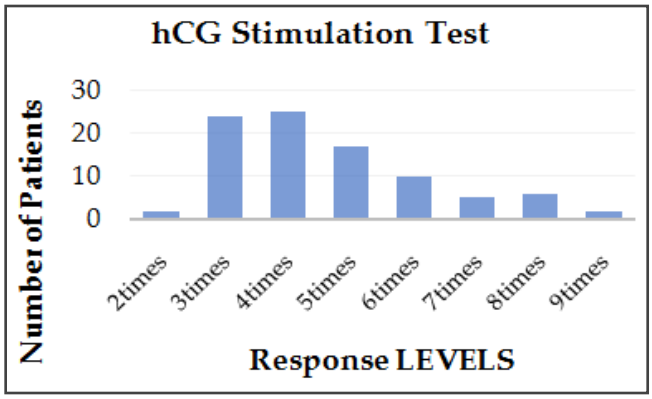

Figure-2: Different response of hCG stimulation in patients with AIS.

post hCG testosterone response. For diagnosis of AIS post hCG testosterone response would be 2 to 9 times of basal testosterone level. Twenty four out of ninety one showed response of 3 times of basal testosterone level and twenty five patients showed 4 times of basal testosterone level.

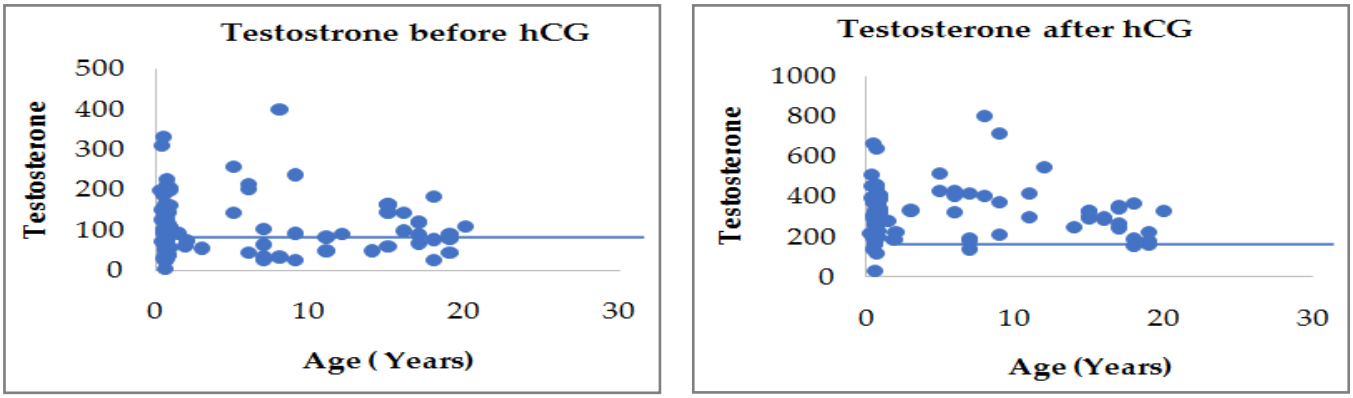

Figure-3: Serum testosterone at different ages before and after hCG stimulation.

DHT and T/DHT Ratio

Serum DHT was analysed in ninety one patients of AIS and T/DHT was also calculated Levels of serum DHT at different age groups are shown in fig-4.

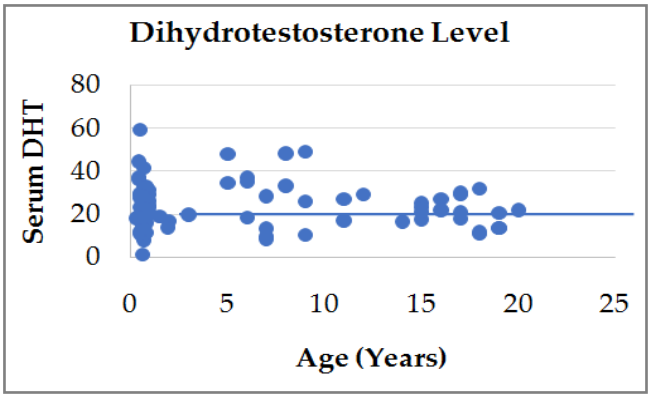

Figure-4: Serum DHT at different ages in patients of AIS.

DISCUSSION

Establishment of diagnosis of AIS is a very difficult job but can be reached by thorough family history of X linked pattern of inheritance, a detailed clinical examination and investigation to rule out other causes of undervirilization. Our study of biochemical investigations, including values of FSH, LH (gonadotropins) and testosterone, DHT (gonadal), have focused on children and adults of various ages. Due to unavailability of gene analysis for diagnosis, AIS was diagnosed by hCG stimulation test. Serum LH, FSH, Testosterone and dihydrotestosterone concentrations are measured, are interpreted in patients with AIS. Therefore we analyzed data of patients of different cities of Pakistan who reported with ambiguous genitalia at Armed Forces Institute of Pathology Rawalpindi. Due to same molecular defects of complete AIS and partial AIS, both are placed in same study group.

On the basis of ages, different doses of hCG is given and its response is observed and analysed. After single dose of hCG, its response to stimulate testosterone is collected from all patients. Adequate response (2-9 times of basal testosterone) of post hCG is suggestive of AIS. A patient with undervirilization and inadequate post hCG testosterone response suggest a defect in testosterone biosynthesis. In our study patients of AIS with undervirilization show adequate post hCG testosterone response. Growth of phallic size in undervirilized patients with post hCG testosterone response is also direct estimation of androgen responsiveness[11].

In literature it is mentioned that In cryptorchid male, inadequate post hCG response is observed as compared to normal physiological male. But no difference in post hCG testosterone

is observed in patients with bilateral scrotal and bilateral abdominal testes.

An inadequate rise in testosterone on hCG stimulation in patients[12] with male under-masculinisation suggests a defect of testosterone biosynthesis but our study demonstrates that in these cases AIS with androgen receptor (AR) abnormalities still need to be considered.

During childhood testosterone level is within reference interval but in child with AIS testosterone concentration is abnormally high and this abnormal surge varies in magnitude in different patients of AIS and in patients with complete AIS and partial AIS[13].

Previous studies have suggested that the SHBG decrement following hCG stimulation should be insignificant in those cases where there is only a minimal rise in testosterone Normally, rise of sex hormone binding globulin (SHBG) concentrations is consistent with testosterone during the early infancy surg and the free androgen index, ratio of testosterone to SHBG, does not change remarkably. However, this ratio increase after administration of exogenous testosterone due to decreased in SHBG concentration and this response has been found to be absent in patients with AIS[14].

In cryptorchidism, lower response to hCG observed, was due to primary testicular defect, mentioned in previous studies. Baseline and post-hCG measurements of DHT were not often performed. Range of T/DHT which was observed in our study is 10.1 to 20 in patient with AIS which is consistent to previous studies in which this range is 1.5 to 17[15].

In our study most of the patient with androgen insensitivity syndrome (AIS) presented with raised serum luteinizing hormone (LH) level. Another interesting feature in AIS patients in our study is that majority of patients had testosterone to dihydrotestosterone (T/DHT) ratio between 10.1-20 and response hCG stimulation was 2-9 times of basal value. Same results were seen in a study done by Chen *et al*[16].

In our study serum LH and product of LH and testosterone- androgen index is raised in patients of AIS which is comparable with a study conducted at Brazil which showed the same results[17].

In one study, It is mentioned that serum LH values were usually above the reference range and the pattern of changes observed in values of LH was similar to that seen in concentration of testosterone[18]. In few cases of AIS, however, serum LH concentration was not greatly raised, demonstrating that high serum LH is not always a marker of AIS.

Interpretation of all investigations which are mentioned in our study is helpful to determine the gonadotropins-gonadal axis in suspected patients of AIS. However, 2-9 times response of post hCG testosterone level exclude testicular biosynthetic defects[19].

Table: Mean (Ranges) of fertility profile of patient of androgen insensitivity syndrome.

Variables	1 day-1 year	1 – 10 years	10 – Onward	*p*- value
LH (IU)	6.80 (5.40-8.70)	6.74 (5.70-7.90)	6.6 (4.70-7.60)	<0.01
FSH (IU)	9.72 (0.24-12.10)	9.07 (0.56-11.80)	10.01 (5.60-12.10)	0.000
Testosterone before hCG (ng/dl)	107.32 (5.40-333.19)	120.76 (26.25-400.99)	98.32 (26.99-453.11)	<0.01
Testosterone after hCG (ng/dl)	310.39 (32.57-666.38)	354.71 (140.52-800.84)	293.43 (155.66-547.75)	<0.01
Dihydrotestosterone Pg/ml	22.72 (1.69-59.24)	26.32 (8.87-49.13)	21.59 (11.11-32.12)	<0.01
hCG response (X basal testosterone)	3.55 (1-8)	4 (2-8)	3.5(2-7)	<0.01
T/DHT	13.65 (7.00-19.58)	13.99 (7.00-19.39)	13.84 (7.00-18.71)	<0.01

Our study has some limitations. Due to a lack of patient outcome data, the predictive value of hCG stimulation test versus T/DHT could not be assessed. Moreover, our study sample consisted of individuals who presented to Endocrine Clinic of AFIP for investigations of disorder of sex development (DSDs) and were not true representative of the population.

RECOMMENDATIONS

A multicenter prospective study should be undertaken to confirm causality between cut off values of fertility profile and AIS in various ethnic groups of Pakistan.

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CONCLUSION

This study find out that cut off of fertility profile of patients at different age groups were determined which would be quiet helpful in diagnosis of patients, with XY karyotype, suspicious of AIS presented with ambiguous genitalia at tertiary care hospital.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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