46 XY DISORDER; ETIOLOGICAL CLASSIFICATION OF THE PATIENT WITH SEX DEVELOPMENT
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ABSTRACT… Objectives: The Disorders of Sex Development are classified as 46, XY DSD, 46, XX DSD and Chromosomal DSD according to the chromosomal constitution of the affected persons. 46, XY DSD is further classified into Androgen Synthetic Defect, Androgen Insensitivity Syndrome Gonadal Dysgenesis, 5-Alpha Reductase Deficiency, Persistent Mullerian Duct Syndrome and Isolated Hypospadias according to the pathophysiology of the disease. The aim of present study was to classify 46, XY patients into their subclasses on the basis of their hormonal profile and physical examination. Study Design: Observational descriptive study.

Setting: Biochemistry Department University of Health Sciences for Karyotyping and Genetic assessment, and its allied institution Biochemistry Department Quaid-e-Azam Medical College Bahawalpur for hormonal analysis, along with Pediatric Medicine Departments of Quaid-e-Azam Medical College / Bahawal Victoria Hospital Bahawalpur for collection of Sample and clinical assessments. Period: June 2015 to December 2015. Study Design: Observational descriptive study. Material and Methods: 53 patients with 46, XY DSD were recruited. Complete clinical history and data of each patient was recorded in the research proforma. Genitals examined for the phallus length and size, position of urinary meatus, palpation of gonads and shape of the labioscrotal folds. Ultrasonography examination of each patient was performed to look for undescended testes and for the presence of either male or female internal reproductive organs.

Results: Base line levels of serum Testosterone Dihydrotestosterone Luteinizing hormone, Follicle stimulating hormone, 17-OH-Progesteron and Anti-mullerian hormones were measured by ELISA technique. Testosterone and DHT were measured again after hCG stimulation. On the basis of physical examination, ultrasonographic findings and hormonal profile diagnosis of the types of 46, XY DSD was possible in 27 (51%) of patients. Androgen synthesis defect as a cause of 46, XY DSD was diagnosed in 7(13%) patients, Androgen insensitivity syndrome in 6(11%) patients, 5-Alpha reductase deficiency in 3(6%) patients, Gonadal Dysgenesis in 3 (6%), Persistent Mullerian Duct Syndrome in 3(6%) and Isolated Hypospadias in 2 (4%) patients. There were 26 (49%) patients which remain undiagnosed with the algorithm of diagnosis used in the present study.

Key words: Disorder of Sex Development, Testosterone, Dihydrotestostosterone, Luteinizing Hormone, Follicle Stimulating Hormone, Gonadal Dysgenesis, Androgen Insensitivity Syndrome.

INTRODUCTION
Disorders of Sex Development are a variety of disorders presenting at birth with atypical genitalia or at puberty as atypical sexual development. In these conditions development of chromosomal, gonadal or anatomical sex is atypical. Previously different confusing terms were used to describe this condition of sexual ambiguity, like intersex, pseudo-hermaphrodite, hermaphrodite etc. These terms do not clearly explain the conditions associated with sexual ambiguity.¹ Keeping in view the problems faced during the diagnosis, and sex assignment in these disorders a new classification system and nomenclature was described in the “Inter-national Consensus Conference on Inter-sex” held in Chicago in the year 2006.² The term Disorder of Sex Development (DSD) was agreed upon to replace the older terms used for sexual ambiguity.² The Disorders of Sex Development are classified as...
46, XY DSD, 46, XX DSD and Chromosomal DSD according to the chromosomal constitution of the affected persons. 46, XY DSD is further classified into Androgen Synthetic Defect, Androgen Insensitivity Syndrome Gonadal Dysgenesis, 5-Alpaha Reductase Deficiency according to the pathophysiology of the disease. The cause of sexual ambiguity in 46, XY, DSDs may be due to deficiency of androgens (gonadal dysgenesis, abnormal synthesis of androgens, deficient 5α-reductase activity), or insensitivity (defect in androgen receptor activity). Androgens, the most important of which is Testosterone, are steroid hormone that are required for the development and maintenance of normal male characteristics. These hormones are essential for the normal differentiation of male internal and external genitalia. Deficiency of these therefore results in ambiguous or female like genitalia in XY fetus. First reason of deficiency is the gonadal dysgenesis, i.e. the sertoli and Leydig cells of testes are not developed resulting so result in absence or decreased production and testosterone hormones secreted by these cells respectively. Mutation in or complete deletion of SRY gene, duplication of dosage sensitive sex locus (DSS) on X chromosome or mutation in autosomal genes are also responsible for this disorder. Secondly the inability to produce testosterone results from defects in any of the enzymes required to synthesize testosterone from cholesterol. The enzymatic defects in testosterone synthesis may be due to deficiency of acute steroidogenesis regulatory protein (STAR), 3β-hydroxysteroid dehydrogenase (3β-HSD) type II deficiency, CYP17 deficiency or P450c17 deficiency. The active form of testosterone is the dihydrotestosterone. Testosterone is converted to its active metabolite, dihydrotestosterone (DHT) in the target tissue by the enzyme 5-alpha-redutase. The mutations in the steroid 5α-reductase type 2 (SRD5A2) gene leads to deficiency of the 5α-reductase. Most of the patients having this deficiency are reared as females due to impairment in the virilization of external genitalia. The aim of present study was to identify the type of disease according to the pathophysiology i.e. the hormonal defect present in patients presenting with disorders of sex development and having 46, XY karyotype and to classify them if possible according to their hormonal profile. This is important for the proper diagnosis and better management of these disorders.

MATERIALS AND METHODS
The observational descriptive study was conducted in Biochemistry Department University of Health Sciences for Karyotyping and genetic assessment, and its allied institution Biochemistry Department Quaid-e-Azam Medical College Bahawalpur for hormonal analysis, along with Pediatric Medicine Departments of Quaid-e-Azam Medical College / Bahawal Victoria Hospital Bahawalpur for collection of Sample, after approval from the ethical committee for Human Research, and Advance Studies and Research Board University of Health Sciences from June 2015 to December 2015. After taking written informed consent from the patient/parents who agreed to participate in the study samples of total number of 53 patients with 46 XY DSD were taken. Children born with ambiguous sex, having age ranging from newborn to 18 years and 46, XY karyotype on chromosome analysis were included in the study while 46, XX karyotype on chromosome analysis and sex chromosomes diseases (Turners, Klinefelter’s Syndromes) were excluded from the study. Data form was filled which comprised of the detailed demographic information about the child. A complete clinical and family history of the child with special reference to the presence of DSD in other family member was also taken. Physical examination of each child was done which included phallus size, phallus length, site of urinary meatus, labioscrotal fusion, right and left gonads, hypospadias, distribution of pubic hair, distribution of axillary hairs, breast development. Ultrasound of each child was done to assess the presence of mullerian or wolfian internal reproductive organs and to detect presence of testis in the inguinal or the abdominal region. After all the clinical procedures blood sampling was done in the laboratory. 5 ml of blood sample was collected by vene puncture in vacutainer without additive.
Serum was separated from cells by centrifugation at the rate of 3000 rpm for 5 minutes. The serum was stored at -80°C until analyzed. For checking hCG stimulation injection hCG 500 IU was given intramuscularly for three days on alternate day then blood sample was taken on the 7th day. Hormonal analysis was done through commercially available ELISA kits. The ELISA Kits used during the hormonal analysis were as, Testosterone Kit (CAT No.DK0002) and FSH Kit (CAT No. DK0010) by DiaMetra from Germany and DHT Kit (CAT No.FR E 2700), 17-OH-P Kit (CAT No.FR E 2800), LH Kit (CAT No.FR E 2600) by Labor Diagnostic Nord GmbH & Co (LDN). The data was analyzed using statical software SPSS 18. In basic descriptive characteristics qualitative variables were given in the form of frequencies and percentages whereas the quantitative variables were given in the form of Mean + S.D.

**RESULTS**

**Characteristics of Subjects**

53 de novo samples of disorder of sex development were included in this study. At the time of presentation 37(70%) patients were assigned male sex by the parents and rest of the 16 (30%) were assigned as females. The ages of the patients ranged from 6 days to 18 years with the mean age + SD 4.47 + 5.11 years. All of the patients presented with ambiguous external genital sex.

**Hormonal Profile and Classification on its basis**

Serum Testosterone, Dihydrotestosterone, LH and FSH hormones were measured in all 53 patients. Serum Testosterone levels were found in the range of 0.01 to 12.14ng/dl with the Mean+SD of 2.3+ 3.7 ng/dl (normal range 0.2 – 2.0ng/dl). Normal testosterone levels were seen in 15 (28%) patients, low testosterone levels were seen in 29 (59%) patients and high in 09 (17%) patients.

Dihydrotestosterone hormone levels in these patients ranged from 19.1 to 822 pg/dl with the Mean+SD of 90+277pg/dl (normal range 250 to 990 pg/dl). These levels were normal in 18(34%) patients, low in 35(66%) patients while none of the patient had DHT levels higher than the normal range. The LH levels were in the range of 0.008-9.79 ng/dl (normal levels 1.5 – 9.3ng/dl) with the Mean+SD of 2.63 + 3.16 ng/dl. Levels were normal in 09 (17%) patients, high in 21(40%) patients and low in 23(43%). Serum FSH levels in these patients ranged from 4.16 to 37.12 ng/dl with the Mean+SD of 6.3+ 6.7 ng/dl (Normal range is 1 to 4 ng/dl). These levels were normal in 10 (19%) patients, high in 17(32%) patients and low in 24(45%) patients.

To determine the presence of functioning testicular tissue and to differentiate between AIS and 5ARDS, hCG stimulation test was done. A total of 43 patients (81%) responded to hCG stimulation, and 10 (19%) remained unresponsive. The hCG stimulation is considered positive if absolute testosterone levels reach the upper limit of pre-pubertal range or rise by more than twice the baseline value.12

Normal levels of serum Testosterone, DHT, LH and FSH were found in 15 (28%) patients. All of these patients responded positively to hCG stimulation test. On calculations T/DHT ratio was found to be more than 20 in 2 out of 15 i.e. (13%) patients and these patients were given a diagnosis of 5-Alpha reductase deficiency based on their investigations and physical findings. T/DHT ration was less than 20 in 13 out of 15 i.e. (87%) patients, 3 of these patients, based on their female like external genitalia were given a diagnosis of Androgen Insensitivity Syndrome(2 CAIS, 1 PAIS). 2 patients having hypospadias as the only presenting complaint were given a diagnosis of Isolated hypospadias while no definite diagnosis could be made for the rest of 8 patients and they require detailed physical & laboratory investigations including molecular studies to reach a diagnosis.

High testosterone levels were found in 9 out of 53 patients. Out of these nine patients 4 had low DHT, T/DHT ratio of greater than 20 on hCG stimulation, female like external genitalia and palpable testes in the inguinal region. These 4 patients were therefore given a diagnosis of 5-Alpha reductase deficiency. The 5 other patients...
had normal DHT, T/DHT ratio less than 20, female like external genitalia with two of them having testes in the inguinal region while testes were detected in abdomen by ultrasonography in one patient. Based on these findings these 5 patients were given a probable diagnosis of Androgen Synthesis Defect (All PAIS). This diagnosis need to be confirmed by molecular analysis of Androgen receptor gene.

Low testosterone levels were found in the largest number of these patients i.e. 29/53 (55%). hCG stimulation in these patients resulted in positive response in 19(66%) of these 29 and no response in 10(34%). Serum 17-OH-Progesterone levels were measured in all these 29 patients. Low level of 17-OH-Progesterone were found in 7 (13%) patients. These patients had high LH & FSH levels, having gonads in the inguinal or labioscral region & variable degree of genital ambiguity were given a diagnosis Androgen Synthesis Defect. The exact pathway involved in this, need further investigation. The rest of the 22 patients had normal 17 OH-P. Out of these 22 normal range patients, 3 patients did not respond to hCG stimulation & their gonads were not found in labioscral, inguinal or abdominal region even by radiography studies. These patients were therefore given diagnosis of gonadal dysgenesis. In rest of 19(66%) there was no diagnosis be made.

The most common disease in this study was Androgen Insensitivity Syndrome and no of cases were 08(14%) patients. Second is Androgen Synthetic Defect i.e. total no. 07(13%) then 5-Alphareductase Deficiency Syndrome with 06(11%) no. of cases, Gonadal Dysgenesis 03(6%), and lastly Isolated Hypospadias with 02(4%) no. of cases. 26(49%) no. of cases remained undiagnosed.

**Final Diagnosis**

<table>
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<th>Name of the Disease</th>
<th>No of Cases (53)</th>
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<td>Androgen Insensitivity Syndrome</td>
<td>08(14%)</td>
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<tr>
<td>Androgen Synthetic Defect</td>
<td>07(13%)</td>
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<tr>
<td>5-Alpha Reductase Deficiency</td>
<td>06(11%)</td>
</tr>
<tr>
<td>Gonadal Dysgenesis</td>
<td>03(6%)</td>
</tr>
<tr>
<td>Others (Isolated Hypospadias)</td>
<td>02(4%)</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>26(49%)</td>
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**DISCUSSION**

Disorders of sex development are wide range of conditions with equally diverse pathophysiology presenting at birth or at the onset of puberty. In newborn with DSD the development of external genitalia is ambiguous making it confusing to assign a proper gender to the baby while in adolescents, failure to achieve sexual maturity or atypical sexual development prompts the patient and their parents to seek medical advice. Having a newborn with DSD is a social stigma for the parents causing anxiety, embarrassment and uncertainty. Similarly atypical sexual development in the adolescents cause psychological trauma to the patients as well as their parents.

The present study was conceived with the aim to establish a diagnostic algorithm for the diagnosis of 46, XY DSD patients by measuring levels of their hormones considered to be involved in differentiation and development of male internal and external reproductive organs. Only 46, XY DSD patients were selected in the present study because in these patients there is heterogeneous presentation and diverse pathophysiology of the disease. On the other hand it is known that in 46, XX DSDs, Congenital adrenal hyperplasia is the most common cause of DSD.12

Physical examination, sex chromosome analysis by karyotyping, pelvic ultrasonography and hormonal analysis are the essential steps in establishing a diagnosis of DSD. In the present study only those patients were recruited who had normal 46, XY karyotype on chromosome analysis.

Based on clinical examination and hormonal profile Androgen insensitivity syndrome was found to be the cause of DSD in majority of patients (14%) in the present study. 3 out of the 8 patients in current study with AIS had normal levels of serum testosterone while 5 had levels higher than the reference values. DHT levels and LH levels were increased in all the patients and all had normal T/DHT ratio of \( \leq 20 \). 2 of these patients, 16 and 18 year of age, were raised as girls and presented to the clinic with the complaint
of primary amenorrhea. They had female-like external genitalia and testes were present in the inguinal region in one patient and were detected in the abdomen in the other. Normal breast development according to age were present in both the patients. Rest of the 6 patients (age from 4 years to 8 years) presented with testes in inguinal region and suspicion of inguinal hernia. 4 of them had perineal hypospadias with under-virilized phallus and were given a diagnosis of PAIS. Remaining 2 patients had female-like internal genitalia with no Mullerian structures on ultrasonography and were also diagnosed as PAIS. The percentage of patients with CAIS (4%) in the present study coincides with the percentage found in study from India (8.7%) and Turkey (7%) while the percentage of PAIS patients in these studies was much higher, 19.2% and 31% respectively against 10% in the present study. Study from Saudi Arabia reported CAIS to be present in 14.28% of their patients while PAIS was reported in 10.7% patients. Study from Indonesia did not demarcated the patients of AIS into CAIS or PAIS but found AIS in 33% of their patients.

Androgen synthetic defect was found in 13% of patients in our study. Comparing Al-Jurayyan in 2011 reported androgen synthesis defect in 14.3% of 46, XY DSD patients from Saudi Arabia. This percentage is similar to the one found in the present study. While in a study from Turkey no patient of androgen synthetic defect was found in 45 patients of DSD nor was any patient with this defect was found in study from Indonesia. Similarly a study from India found androgen synthesis defect in 7.1% of DSD patients. Patients with androgen synthesis defect in the present study had low testosterone levels, low 17-OH progesterone levels, low response to hCG stimulation and increased FSH and LH levels. These patients had underdeveloped male internal reproductive organs on ultrasonography scan. In the present study only a broader diagnosis of Androgen synthetic defect could be made as testosterone is produced from cholesterol through a number of biochemical conversions and defect in any of the enzymes needed for these conversions results in defect in androgen production despite the presence of testes. There could be defect in Steroidogenic acute regulator (StAR) protein, P450 side chain cleavage/CYP11A1 enzyme deficiency or 17α-Hydroxylase/17,20 Lyase deficiency. Lydig cell hyperplasia or LH receptor defects may also result in androgen biosynthesis defect. To identify the exact cause of defect in androgen synthesis in the present study requires further biochemical and molecular investigations.

5-Alpha reductase deficiency was found in 6 (11%) of patients in the present study. All these patients had normal testosterone levels, decreased DHT levels and markedly elevated serum T/DHT ratio ≥ 20. This elevated ration is the hallmark of the diagnosis of 5-Alpha reductase deficiency. All these patients had perineal hypospadias with three having unilateral undescended testes while two had bilateral testes in the inguinal region. Three of the patients had female-like phallus while two had micropenis. Labioscrotal folds were unfused in all the patients. 5-Alpha reductase enzyme is expressed by SRD5A2 gene present on chromosome 2 and this enzyme catalyzes the conversion of testosterone into the more potent androgen, dihydrotestosterone. When compared with studies from other populations, the percentage of patients diagnosed with 5-Alpha reductase deficiency (10%) in the present study is closer to that from Turkey (7%) and Saudi Arabia (14.28%). Study from India has reported a very high percentage (22%) of 5-ARDS.

Gonads were not palpable nor detected by ultrasonography scan in 3 (6%) patients. These three patients had low levels of serum testosterone and DHT, raised LH and FSH levels and no response to hCG stimulation test. These patients were therefore diagnosed as having Gonadal dysgenesis. Gonadal dysgenesis in 46, XY individuals is a heterogeneous group of disorders resulting from mutations or deletions in testes determining gene SRY or duplication of dosage specific sex (DDS) locus on X-chromosome. Mutations in many autosomal genes have also been reported in cases of gonadal dysgenesis. All of the patients diagnosed in the present study as having Gonadal dysgenesis were raised as
females and had female like external genitalia. One of the patient had underdeveloped Mullerian structures while no Mullerian structure was found on ultrasonography in rest of the two patients. The percentage of patients with gonadal dysgenesis in the present study is lower than that reported from Indonesia, India and Turkey, 16%, 16% and 13% respectively.13,1,15

Finally two (4%) of the patients had peno-scrotal hypospadias with normal male like phallus and testes located in normally formed scrotum. They had normal hormonal profile and normal internal male reproductive organs. These patients were therefore labelled as having isolated hypospadias. X-linked recessive, autosomal dominant and autosomal recessive modes of inheritance has been reported in patients with isolated hypospadias.21 No definite diagnosis could be given to the remaining 49% of patients in the present study. This was due to very heterogeneous and complex presentation and not very informative hormonal profile. It has been reported in the literature that 30-40% of cases with 46, XY DSD remain undiagnosed despite extensive diagnostic workup.5

CONCLUSION

The present study was conducted to define a possible algorithm for a stepwise approach to reach the diagnostic classification of patients with DSD. Based on the algorithm a probable diagnosis was possible in 51% of patients. Addition of more biochemical tests like precursors of Testosterone biosynthesis precursors would further increase the usefulness of the proposed algorithm. Based on current information and limitations of the biochemical analysis it justified to incorporate molecular genetic studies in the diagnostic algorithm for the diagnosis of DSDs. Additional studies are required to identify the more useful and cost effective approach to the diagnosis of local patients with DSD so that an early and specific management plan leading to optimal outcome could be designed for them.

REFERENCES


46 XY DISORDER

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**AUTHORSHIP AND CONTRIBUTION DECLARATION**

<table>
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<td>Dr. Saqib Mehmood</td>
<td>Karyotyping, Collection of literature and help in writing Discussion</td>
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