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Study of 5 Alpha Reductase Enzyme Gene Mutations in a Group of Patients with 46XY Disorders of Sex Development

Thesis

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List of Abbreviations

Abb.	Full term
5α-DHP	5α-dihydroprogesterone
AIS	Androgen insensitivity syndromes'
AMH	Anti mullerian hormone
AR	Androgen receptor
CAIS	Complete Androgen Insenstivity
CGD	Complete gonadal dysgenesis
CT	Computed tomography
CYB5	Cytochrome b5
ddx	Differential diagnosis
DEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DSD	Disorders of sex development
EMS	External masculinization score
FSH	Follicle-stimulating hormone
HCG	Human chorionic gondatropin
HS	Highly significant
IQR	Interquartile range
LBD	Ligand binding domain
LH	Luteinizing hormone
MAIS	Mild androgen insensitivity
NS	Non-significant
PAIS	Partial androgen insensitivity
PCOS	Polycystic ovary syndrome
PGD	Partial gonadal dysgenesis
PMDS	Persistent mullerian duct syndrome
POR	P450 oxidoreductase
S	Significant
SDS	Standard deviation score
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package for Social Sciences
STAR	Steroidogenic acute regulatory protein
XLAG	X-linked lissencephaly and ambiguous genitalia

Study of 5 Alpha Reductase Enzyme Gene Mutations in a group of Patients with 46XY Disorders of Sex Development

ABSTRACT

Background: Disorders of sex development (DSD) are a wide range of conditions with diverse features and pathophysiology that most present in the newborn or the adolescent periods. Affected patients usually present with atypical genitalia like hypospadias, isolated micropenis and undescended testes. These clinical situations can often be difficult to manage, particularly in those cases where the sex rearing is uncertain.

Aim of the work: Studying 5alpha reductase enzyme gene mutations in patients with 46XY DSD and the use of EMS in patients with 46XY DSD will help to assess the degree of virilization.

Patients and Methods: This cross-sectional study was performed on 25 male patients with clinical, laboratory and genetic criteria fitting the diagnosis of 46XY DSD. All parameters were evaluated at the pediatrics endocrinology clinic and genetics department, Ain Shams University. The duration of study was 1 year from December 2019 to December 2020.

Results: Our results showed Mean EMS of 4.3 denoting the high degree of genital ambiguity in our cases. Regarding type of mutations 56.2% of mutations were Insertion deletion, 25% of mutations were Insertion, 12.5 % were Missense, 6.25% of mutations were Deletion. While phenotype–genotype correlation for 5αR deficiency has not been well established, the reason for such variability is unclear.

Conclusion: This study demonstrated Ten novel mutations of *SRD5A2* gene were identified in association with 5ARD2. These mutations were reported among 12 patient patient with 46 xy DSD from total 25 patients were fit for the thesis.Mean EMS score among those patient was 4.3 .

Keywords: 5 Alpha Reductase Enzyme Gene, 46XY Disorders, Sex Development , EMS.

INTRODUCTION

Disorders of sex development (DSD) are a wide range of conditions with diverse features and pathophysiology that most often present in the newborn or the adolescent periods. Affected patients usually present with atypical genitalia like hypospadias, isolated micropenis and undescended testis. These clinical situations can often be difficult to manage, particularly in those cases where the sex rearing is uncertain ***(Maimoun et al., 2011).***

Developing a logical plan for investigations while establishing a dialogue for parents is central to the initial approach and ongoing management ***(Biason-Lauber, 2010).***

Many gene Mutation have been discovered like POR, HSD3B2, HSD17B3, CYP17A1, MAMld1, MAMLD1, CBX2, 5alpha reductase in DSD cases ***(Hughes et al., 2006).***

The 5-alpha reductase enzyme is responsible for converting testosterone to its more active form, dihydrotestosterone (DHT). DHT plays an important role in the development of external male genetlia; its deficiency is inherited in an autosomal recessive pattern and it is caused by mutations in the SRD5A2 gene which encodes for it. The patient will have either normally looking female external genitalia to completely ambiguous genitalia ***(Cheon, 2011).***

To avoid unnecessary irrelevant investigations, patients must be examined using external masculinization score (EMS). Calculating the EMS provides an objective aggregate score of the extent of masculinization of the external genitalia. Each feature of the genitalia (phallus size, labioscrotal fusion, site of the gonads and location of urethral meatus) can be individually scored to provide a score out of 12. EMS less than 7 is considered ambiguous ***(Lek et al., 2014).***

AIM OF THE WORK

Studying 5alpha reductase enzyme gene mutations in patients with 46XY DSD. The use of EMS in patients with 46XY DSD will help to assess the degree of virilization.

DISORDERS OF SEX DEVELOPMENT

Disorders of sex differentiation (DSD) occurs if there is any discordance between (chromosomal, gonadal, phenotypic sex determination). Terminologies such as hermaphrodite, pseudo-hermaphrodite, and intersex, are considered to be old and outdated.

The birth of an infant with ambiguous genitalia generates difficult multiple medical, surgical, ethical, psychosocial, and physical issues for patients and their families. Phenotypic sex is determined by the differentiation of internal ducts and external genitalia under the influence of hormones and other additional factors ***(Hughes et al., 2007).***

There are two major processes during normal sexual development. The first of which is sex determination in which the bipotential gonads are induced to form either male testes or female ovaries. The second occurs under effect of hormones formed by the newly differentiated gonand to regulate the formation of internal and external genetalia ***(Arboleda et al., 2014).***

Table 1: Revised terminology for DSD

Old Terminology	New Terminology	Description
Intersex	DSD	Development of genitalia is abnormal
True hermaphroditism	Ovotesticular DSD	Both ovarian and testicular tissues are present; internal and external genitalia are ambiguous
XY sex reversal (XY female sex)	Complete gonadal dysgenesis	Streak (nonfunctional) gonads as well as müllerian structures are present; external genitalia are female
XX sex reversal (XX male sex), female pseudohermaphroditism	46,XX testicular DSD	Testes are present; internal and external genitalia are male
Male pseudohermaphroditism, XY male undermasculinization	46,XY DSD	Male gonadal development is abnormal; androgen synthesis or action is deficient; external genitalia are undermasculinized to a variable degree
Female pseudohermaphroditism, XX female overvirilization, XX female masculinization	46,XX DSD	Female gonadal development is abnormal; androgen synthesis or action is excessive; external genitalia are masculinized to a variable degree

(Hughes et al., 2006)

Development of Normal male gonads:

There are three sequential stages necessary for normal gonadal development. The first is the undifferentiated stage where, identical primitive structures develop in both XY and XX embryos. The second is gonadal determination where ovaires or testes develop from bipotential gonads. Third stage involves development of both internal and external genetalia either to male genetalia in presence of testicular hormones, or female genetalia in their absence ***(Delot and Vilain, 2019).***

I. Undifferentiated Stage of Sexual Differentiation

In the undifferentiated stage, gonads are bipotential, and Mullerian and Wolffian ducts are present in both XX and XY fetuses. The urogenital sinus is also bipotential. Existence of testis

and its hormones leads to male reproductive tract from wolffian duct and regression of mullerian duct. In case of testicular absence, Mullerian duct gives rise to female reproductive tract whether ovaries are present or not and Wolffian duct degenrates ***(Delot and Vilain, 2019).***

II. Gonadal differentiation

SRY present only in the XY gonadal ridge is the main factor for testicular differentiation from the gonadal ridge. Genetic expression of certain pattern mainly regulates normal differentiation of the gonadal ridges into either testes or ovaries ***(Zhao et al., 2017).***

III. Male Differentiation of the internal and external Genitalia

1. The determining role of testicular hormones

Adequate testicular hormones production and function are essential for internal and external genital differentiation in a male pattern; and the absence of testes results in female genital differentiation, whether ovaries exist or not ***(Rey et al., 2016).***

2. Anti mullerian hormone (AMH) and Mullerian duct regression

Regression of mullerian ducts occurs mainly under effect of glycoprotein hormone called AMH. Androgens are potent inhibitors of AMH expression at puberty. Absence of androgen receptor expression in sertoli cells during fetal and early postnatal

life explains the high levels of both AMH and androgens ***(Mehmood and Rentea, 2020).***

3. Androgens and INSL3: virilisation of internal and external genitalia and testicular descent

Leydig cells are responsible for testosterone production from cholesetrol through the action of steroidogenic enzymes, many of which are regulated by SF113. Initially, Sertoli- and peritubular cell-secreted paracrine factors, like DHH helps in Leydig cell differentiation. However, placental human chorionic gondatropin (HCG) and fetal pituitary lutenizing hormone (LH), both acting through the same LH/hCG receptor are the major regulators of Leydig cell stabilization and final differentiation afterwards. hCG action is essential in the first trimester when the fetal hypothalamic-pituitary axis is not functional yet. After mid-gestation, LH takes over ***(Khattab et al., 2020).***

Wolffian duct differentiation into the epididiymis, vas deferens and seminal vesicle takes place under effect of testosterone in the first trimester. High testosterone concentrations needed for normal male gonaduct differentiation. which can only be supplied by the neighborhood of the homolateral testis. The Wolffian duct degenerates in absence of testosterone action ***(Baetens et al., 2019).***

Testosterone is converted by 5α-reductase to dihydrotestosterone (DHT), the active and more potent form. Virilisation of the urogenital sinus and external genitalia occurs