Anti-Müllerian Hormone Levels in Girls and Adolescents with Turner Syndrome: Relation to Karyotype, Pubertal Development and Growth Hormone Therapy

Thesis

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Traiz Ezzat Riad

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List of Abbreviations

Abb.	Full term
AFC	Antral follicle count
ALKs	Activin receptor-like protein kinases
AMH	Anti-Müllerian hormon
AMHRll	AMH one type II receptor
ANOVA	Analysis of variance
APCD	Anterior-posterior cervical uterine segment diameter
APFD	Anterior-posterior fundal segment diameter
BMD	Bone mineral density
BMPs	Bone morphogenetic proteins
CHL	Conductive hearing loss
Co A	Co arcitation of the aorta
E2	Estradiol
ERT	Estrogen replacement therapy
FSH	Follicle stimulating hormone
GH	Growth hormone
GHD	Growth hormone deficiency
GnRH	Gonadotropin releasing hormone
HH	Hypogonadotropic hypogonadism
HPG	Hypothalamo-pituitary gonadal
HRQOL	Health-related quality of life
IBD	Inflammatory bowel diseases
IGF	Insulin like growth factor
KS	Klinefelter Syndrome

List of Abbreviations (Cont.)

Abb.	Full term
LH	Luteinizing hormone
Lt	Left
MRI	Magnetic resonance imaging
OHSS	Ovarian hyper stimulation syndrome
Ov	Ovarian
P	Probability
PAR	Pseudoautosomal region
POI	Premature ovarian insufficiency
POI	Primary ovarian insufficiency
RFLP	Restriction-fragment length polymorphism
r-hGH	Recombinant human GH
Rt	Right
SCFE	Slipped capital femoral epiphysis
SD	Standard deviation
SDS	Standard deviation score
SHOX	Short stature homeobox gene
SNHL	Sensorineural hearing loss
SNP	Single nucleotide polymorphism
TGF-II	Transforming growth factor-II
TS	Turner syndrome
tTg	Tissue transglutaminase
US	Ultrasound
Vol	Volume
XM	Maternal X chromosome
XP	Paternal X chromosome

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INTRODUCTION

Turner syndrome (TS) is found in 1:2000 newborn girls. The classic form is associated with the 45X karyotype (50%), whereas variants either show a mosaic of 45X/46XX (25%) or have structural anomalies of the X chromosome such as deletions, iso-or ring chromosomes (25%) ***(Saenger et al., 2001 and Bondy, 2007)***. The clinical spectrum of TS is broad and highly variable but short stature and gonadal dysgenesis are characteristic features. Gonadal dysgenesis in TS results in pubertal delay or failure and infertility in most patients. However, up to 30% of girls with TS have spontaneous pubertal development and 2–5% have regular menstrual cycles before the onset of premature menopause ***(Abir et al., 2001)***. Spontaneous pregnancies occur only in 2% of women with TS, mostly at a young age (23–24 years) and appear to be associated with the mosaic karyotype ***(Hovatta, 1999)*.**

Primary ovarian insufficiency (POI) in patients with TS is due to an accelerated loss of follicles from the ovaries, which may start as early as 18 weeks into fetal life ***(Weiss, 1971; Santoro, 2003 and Reynaud et al., 2004)***. More recent studies found that there may be viable follicles even in the ovaries of 12–13 years old girls with classical TS without spontaneous pubertal development ***(Gravholt et al., 2002 and Hreinsson et al., 2002)***, although the quality of these follicles is doubtful. Thus, preservation of fertility in patients with TS may be feasible through cryopreservation of ovarian tissue before

follicles begin to disappear ***(Rutherford and Matthews, 2000 and Huang et al., 2008)*.**

However, to determine the possible candidates among girls and young adolescents with TS and the right time point for such interventions, biochemical markers reflecting the ovarian reserve in childhood are needed. Antral follicle count (AFC) and early follicular phase serum levels of FSH, inhibin B and estradiol (E2) are measured to assess a woman's ovarian reserve ***(Burger et al., 1995)*.**

However, all methods that assess the ovarian reserve are based on the mature, adult female hypothalamic-pituitary-gonadal axis. In contrast, gonadotropin-releasing hormone (GnRH) is switched off after birth and remains silent until the age of 10 years when healthy girls start puberty. During this time FSH, inhibin B and E2 levels are low. Although gonadotrophin levels (FSH and LH) tend to be higher in patients with TS, especially in the first 2–5 years of life, a significant overlap exists between TS and normal girls especially during mid-childhood. Therefore, gonadotrophin levels may not reflect the ovarian reserve before the onset of puberty ***(Conte et al., 1975; Chrysis et al., 2006 and Hagen et al., 2010b)***.

In the past, we and others have shown that the serum anti-Müllerian hormone (AMH) level is a good marker for the size of the growing follicle pool, and, indirectly, also of the primordial follicle pool, reflecting the ovarian reserve ***(de Vet***

***et al., 2002; van Rooij et al., 2002; vanRooij et al., 2005; Pignyet al., 2006 and Visser et al. 2006)*.** AMH is specifically expressed in granulosa cells of growing non-selected follicles. In women of reproductive age, serum AMH levels correlate strongly with AFC and levels decline over time to become undetectable at menopause. Recent studies in healthy normo-ovulatory adult women confirmed this decline in serum AMH with increasing age ***(Kelsey et al., 2011 and Lie Fong et al., 2012)*.**

Interestingly, this decline in serum AMH levels precedes the changes in traditional markers for ovarian reserve, such as FSH, inhibin B and E2. Hence, it is believed that serum AMH levels constitute an ovarian reserve marker in adult women, independent from the hypothalamo-pituitary gonadal (HPG) axis ***(Borgstrom et al., 2009 and Hagen et al., 2010a)*.**

During childhood AMH levels increase slightly from birth onward and plateau during adolescence, suggesting that follicle dynamics in children may differ from that in adults. Not until the age of 25, the decline in serum AMH with increasing age is observed ***(Hagen et al., 2010a; Kelsey et al., 2011 and Lie Fong et al. 2012)*.** Nevertheless, assessment of serum AMH, also at a young age, is indicative of ongoing follicular development ***(Visser et al., 2012)*.**

AIM OF THE WORK

The aim of this study is to:

- Assess serum AMH levels in girls with TS.
- Find out if there is a relation between serum AMH levels and each of karyotype, spontaneous puberty, and growth hormone (GH) therapy in such girls.

Chapter 1

TURNER SYNDROME

Definition and introduction:

Turner syndrome (TS) is diagnosed by the combination of certain phenotypic characteristics with the absence of one of the X chromosome. This absence may be total or partial, as occurs in isochromosomes Xq. The phenotypic consequences of these depend on two factors: the characteristics of the lost genes and the percentage of cells 45, X in mosaicisms. The clinical features also change with the cytogenetic pattern. Short stature is the most common phenotypic manifestation ***(Cuesta et al., 2015).***

It usually accompanied with short stature, gonadal dysgenesis, lymphedema, and characteristic dysmorphic appearance in the severe phenotype, though it has a minimal impact on stature or secondary amenorrhea in the mild phenotype chromosome can include deletions of the short arm and duplication of the long arm to form isochromosome (isoXq) and undergo ring formation (rX) and deletion in the short or long arm (Xp-, Xq- respectively). Some individuals are mosaic and carry one or more additional cell lines, also with the Y chromosome (45, X/46, XX, 45, X/46, XY). As a result, TS patients are haploinsufficient for some genes. The described karyotype variability reflects the wide clinical spectrum of the syndrome **(*Gawlik & Malecka-Tendera, 2014).***

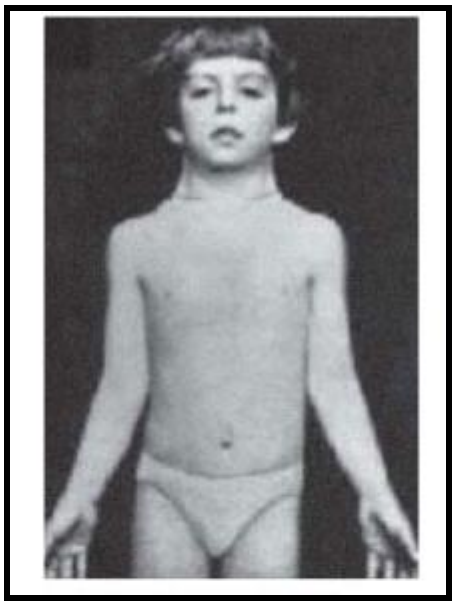

Figure (1): Child with phonotypical characteristics of Turner's syndrome, particularly elbow deformities and webbed neck (Source: http://www.caihand.com/images/ turner.jpg)

History & other names:

TS is named after Henry Turner, he originally described it in 1938 prior to the advent of chromosome analysis, consisted of a constellation of phenotypic findings; short stature, sexual infantilism, webbed neck, and cubitus valgus ***(Turner, 1938).***

It is often called **Ullrich-Turner syndrome** or even **Bonnevie-Ullrich-Turner syndrome** to acknowledge that earlier cases had also been described by European doctors ***(Ford et al., 1959; Zhong and Layman, 2012).***

The first published report of a female with a 45, X karyotype was in 1959 by Dr. Charles Ford and colleagues in Harwell, Oxford shire and Guy's Hospital in London. It was

found in a 14-year-old girl with signs of Turner syndrome. Also known as "Gonadal dysgenesis" ***(Ford et al., 1959; Zhong and Layman, 2012).***

Epidemiology

TS is the most common chromosomal abnormality affecting approximately one in 2500 live-born female ***(Gawlik and Malecka-Tendera, 2014).***

The classic presentation of TS includes short stature with nearly 100% of patients, and delayed puberty in 60-90%.Additional features are quite variable but include edema of hands or feet, webbed neck, low posterior hairline, nail dysplasia, rotated ears, small mandible, nail hypoplasia, hyper convex nails, multiple pigmented nevi, characteristic facies, broad shield chest, cubitus valgus, short fourth metacarpal, and high arched palate. However, the left-sided cardiac anomalies occur in about 50% and renal anomalies seen in one-third, there may also be an increased risk of Hashimoto's thyroiditis and hypothyroidism, particularly if an isochromosome of X is present. These somatic features may be present regardless of the presence or absence of mosaicism or the degree of mosaicism. Approximately 45% of postnatal TS patients have a pure 45, X cell line without any detectable mosaicism. Other karyotypes that may be mosaic with 45, X most commonly include: 46, X, I (Xq), 46, XX, 47, XXX, 46, X, Del (Xp), or 46, XY. Typically the isochromosome of the X, consisting of two long arms of X and designated 46, X, I (Xq), is the most