Human Reproduction Vol.20, No.5 pp. 1418–1421, 2005 doi:10.1093/humrep/deh764
Advance Access publication February 3, 2005

Uterine size in women with Turner syndrome after induction of puberty with estrogens and long-term growth hormone therapy: results of the German IGLU Follow-up Study 2001

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BACKGROUND: To evaluate the factors influencing uterine size in young adult women with Turner syndrome (TS) after long-term growth hormone (GH) treatment. METHODS: Cross-sectional study. Out of 188 women with TS from 96 German centres, whose longitudinal growth was documented within KIGS (Pfizer International Growth Database), data on uterine size were collected voluntarily at a standardized follow-up visit: 75 TS women (ages: 15.8–30.8 years) with complete data were included. Classification according to karyotype: 45,X (78.6%), 45,X/46,XX (5.4%), 45,X/46,iXq (8%), 45,X/46,XY (8%). Puberty was induced with estrogens in all women. At follow-up, 66 were on cyclic estrogens and progestins. RESULTS: 13/66 (19.6%) TS women who received estrogens had a reduced uterine length <5 cm. Calculating the data in standard deviation scores (SDS), only women with 45,X/46,XX karyotype had normal median uterine length and volume of 0.6 and 1.59 SDS respectively. An incomplete breast development (Tanner stage B 3) was found in women with 45,X karyotype (n = 11; 18.6%) and with 45,X/46,XY (n = 2). CONCLUSIONS: Only TS women with karyotype 45,X/46,XX had normal uterine sizes, whereas 26% of the TS women with karyotype 45,X had a uterine length < −2 SDS, and 18% a volume < −2 SDS.

Key words: estrogen therapy/gonadal dysgenesis/Turner syndrome/uterine size

Introduction

Short stature and ovarian failure are the main clinical features in Turner syndrome (TS). The lack of estrogens is reflected by the uterine size which is described as pre-pubertal or intermediate (Shawker *et al.*, 1986; Mazzanti *et al.*, 1997; Haber and Ranke, 1999). Data on the effect of estrogen therapy on uterine size in girls with TS are rare in the literature (Paterson *et al.*, 2002; McDonnell *et al.*, 2003; Snajderova *et al.*, 2003). The question arises whether TS women can achieve a normal uterine size after adequately timed estrogen therapy in puberty using recommended doses.

Thus, our present analysis focused on uterine growth assessed by ultrasonographic imaging in young adult women with TS after induction of puberty with estrogens and therapy with human growth hormone (hGH). The auxological data of the identical women after hGH therapy were reported recently (Ranke *et al.*, 2002).

Materials and methods

The TS women enrolled in this study were derived from a group of 188 women from the German 'Internationale Genotropin Langzeit-Untersuchung' (IGLU) Follow-up Study 2001. The IGLU database, which is the German segment of Pfizer International Growth Database (KIGS), documented girls with TS from 96 German centres. The project was primarily designed to evaluate the results of human growth hormone (hGH) treatment. On hGH treatment (median dose: 0.29 mg/kg per week), the girls were seen regularly every 4–6 months by the participating physicians. The follow-up visit after the end of hGH therapy was conducted according to a standardized protocol approved by the Ethics Committee of the participating institutions, and written, informed consent was obtained at the time of the follow-up visit. Karyotype details were supplied by the referring physicians.

In addition to anthropometric data, data were collected voluntarily for uterine size. Thus, out of a total of 188 TS women returned for examination, complete data on uterine length and volume were

provided for 75. In all, puberty had to be induced with estrogens. For pubertal induction either low-dose ethinylestradiol ($n = 18$), conjugated equine estrogen ($n = 17$) or estradiol valerate ($n = 38$) was used. In two women, a combined oral contraceptive was used to induce puberty. After 12–18 months on estrogens, cyclic progesterone was added to mimic a normal menstrual cycle. At the time of the follow-up study, 66/75 women were on cyclic estrogen and progestin supplementation, $n = 2$ (karyotype 45,X) received no estrogens, and data were missing for $n = 7$ (karyotype 45,X).

The evaluation of uterine size by pelvic ultrasonography was carried out transabdominally within the participating institutions. An illustration of the requested planes was provided to the physicians in order to standardize the measurements of the uterus in the transverse, sagittal and longitudinal planes. Uterine length was reported in cm, and uterine volume was calculated by the formula: volume (ml) = transverse width × sagittal width × length × 0.5. The data on uterine length and volume were transferred into standard deviation score (SDS) values using reference values published by Pelzer for healthy German girls (Pelzer, 1991).

The anthropometric data were analysed according to previously described criteria (Ranke *et al.*, 2000). We applied the height references for TS of Ranke *et al.* (1988). Standard deviation scores for ages were calculated. Pubertal development was assessed according to Tanner puberty stages. Medians and ranges are given, and one-way analysis of variance (ANOVA) was used to obtain P values. $P < 0.05$ was considered significant.

Results

The 75 TS women were classified according to karyotype into four different groups. The majority (78.6%) had the karyotype 45,X, whereas the number of women with other karyotypes was low, as expected from the distribution of the karyotypes from the whole cohort (Ranke *et al.*, 2002). All with karyotype 45,X/46,XY showed a normal female external genitalia, no male structures, a uterus, and had had a gonadectomy due to the risk of gonadoblastoma. Clinical characteristics (median values) at the time of the follow-up visit describing the groups are shown in Table I. There were no differences in terms of age, height, and body mass index (BMI) values. None of the women suffered from serious side-effects of GH therapy during follow-up. In all TS women, puberty was induced with estrogens, but at follow-up data were missing for $n = 7$ with karyotype 45, X. Despite long-term estrogen therapy, an incomplete breast development (Tanner B 3) was found in some young women with 45,X karyotype ($n = 11$; 18,6%) and with 45,X/46,XY ($n = 2$). All TS women with incomplete breast development had been treated with estrogens and were on estrogens at follow-up.

Table II shows parameters such as age at start of estrogens, age at start of cyclic therapy, age at induced menarche, and duration of estrogen therapy (i.e. from start until time of follow-up visit). The parameters were not significantly different between the groups. Data on menarcheal age of women with 45,X/46,XY karyotype were missing. Data on duration of estrogen therapy (45,X group) could be calculated for 50 of the 59 TS women only. Overall, estrogens were started at a relatively late age, thus the girls were relatively old at time of induced menarche.

Uterine measurements are shown in Table I. The data of the 45,X group were calculated only for TS women who received estrogens at follow-up ($n = 50$). Calculating the data on uterine length and volume as SDS values, median length and volume were normal in women with karyotype 45,X/46,XX with 0.6 SDS (length) and 1.59 SDS (volume). Since the other groups were too small, there were no significant differences between the groups. The two women with karyotype 45,X who did not receive any estrogens at follow-up had smaller uterus lengths of 4 cm (−2.6 SDS) and 4.8 cm (−1.8 SDS) respectively, and uterus volumes of 8 ml (−2.2 SDS) and 11 ml (−1.9 SDS).

It has been reported that the uterus measures between 5 and 8 cm after puberty in young healthy nulliparous adolescents (Cohen and Haller, 1989). Comparing our data with these values, 42/50 (84.0%) TS women with karyotype 45,X

Table I. Characteristics of Turner syndrome (TS) women at follow-up according to karyotype

Variables	Groups			
	45,X	45,X/46,XX	45,X/46,X, i(Xq)	45,X/46,XY
n	59	4	6	6
Age (years)	19.7 (16.3–30.8)	19.9 (19.3–24.4)	18.0 (16.1–26.0)	18.4 (15.8–21.1)
Height (cm)	152.6 (141.1–167.3)	148.0 (144.6–152.6)	150.8 (143.5–162.6)	151.4 (148.2–157.5)
Height SDS (Ranke)	1.03 (−0.85–3.44)	0.29 (−0.28–1.03)	0.74 (−0.46–2.61)	0.84 (0.31 − 1.84)
BMI (kg/m^2)	23.7 (17.7–41.5)	27.6 (20.7–36.1)	23.1 (21.8–37.9)	20.1 (19.5–23.5)
Tanner breast stage	B 3 (18.6%)	–	–	B 3 (33.3%)
	B 4 (27.1%)	B 4 (25.0%)	B 4 (50%)	B 4 (16.7%)
	B 5 (54.3%)	B 5 (75.0%)	B 5 (50%)	B 5 (50.0%
Estrogen therapy	84.8	100	100	100
No data (%) ($n = 7$)	11.8			
No therapy (%) ($n = 2$)	3.4			
Uterine length (cm)	5.7[a] (2.8–10.0)	7.2 (5.8–9.0)	5.2 (3.8–6.6)	5.7 (5.0–6.5)
Uterine length (SDS)	−0.70[a] (−3.8–3.4)	0.60 (−0.7–2.4)	−1.35 (− 2.8–0.0)	−0.66 (−1.6 to −0.1)
Uterine volume (ml)	17.5[a] (4.9–70)	32.7 (11–35)	11.4 (7–29)	12.5 (2.3–47)
Uterine volume (SDS)	−1.10[a] (−2.62–5.22)	1.59 (−1.89–1.0)	−1.84 (−2.37–0.28)	−0.95 (−2.94–2.45)

Values are median values, range unless stated otherwise.
[a]Data were calculated for 50/59 TS patients.
SDS = standard deviation score; BMI = body mass index.

Table II. Characteristics of Turner syndrome (TS) women according to induction of puberty (median values, range)

Variables	Groups			
	45,X	45,X/46,XX	45,X/46,X,i (Xq)	45,X/46,XY
n	59	4	6	6
Age (years) at start of estrogens	14.6 (11.8–21.7)	15.2 (14.4–16.9)	15.0 (14.1–17.3)	14.2 (13.1–17.9)
Age (years) at start of progestins	16.1 (12.8–22.8)	16.9 (14.4–19.3)	16.5 (15.0–18.0)	15.2 (14.2–16.6)
Age (years) at menarche	16.2 (12.5–23.9)	16.0 (13.8–19.3)	17.3 (15.1–24.4)	No data
Duration (years) of estrogen therapy	5.3 (1.5–10.7)	5.3 (3.6 –7.4)	1.9 (1.6–4.4)	2.5 (0.8–7.8)

had a normal length, but 8/50 (16%) had a smaller uterus. All four women with karyotype 45,X/46,XX, 3/6 with 45, X/46,X,i(Xq), and 4/6 with 45,X/46,XY had a normal uterine length. Overall, 13/66 (19.6%) who received estrogens had a reduced length <5 cm. With respect to the data published by Pelzer (1991), which we used as reference for SDS calculations, 13/50 (26%) of the TS women with karyotype 45,X had a uterine length <-2 SDS, and 9/50 (18%) a volume <-2 SDS. In order to evaluate uterine size at follow-up, the variables' uterine length and volume were correlated with various parameters during the course of treatment, e.g. age at start of estrogens, age at cyclic substitution, duration of estrogens, and Tanner breast stage for TS girls with karyotype 45,X. No significant correlations between uterine size and the studied parameters were found. Also, final height data available for all patients showed no correlation between uterine size and height.

Discussion

More than 90% of girls with TS have no pubertal development and primary amenorrhea due to primary ovarian insufficiency. Different studies have shown that adolescent or young adult TS women without estrogen substitution had a hypoplastic uterus (Mazzanti *et al.*, 1997; Haber and Ranke, 1999). Shawker *et al.* (1986) reported ultrasound findings in a small group of women with regard to karyotype. TS girls with monosomy had pre-pubertal uterine dimensions, whereas girls with mosaic had uterine lengths ranging from pre-pubertal to adult size. Massarano *et al.* (1989) reported that girls who did not have streak ovaries showed a marked increase in uterine length at the expected age. This stresses the importance of estrogens in the development of uterine size in TS women. Our finding that TS women have a somewhat more pronounced reduction of uterine volume than uterine length confirm previous data, showing that difference in uterus volume is related more to a reduced width rather than abnormal length (Haber and Ranke, 1999).

It was postulated that estrogens given at the age when puberty normally occurs might normalize uterine size in young TS women (McDonnell *et al.*, 2003). Our data contrast with these data. The difference could be explained by the facts that the studied women were younger at last examination than ours, and that 5/18 girls had normal spontaneous puberty, whereas puberty was induced in all our women. Paterson *et al.* (2002) found that estrogen therapy in Turner girls enhanced linear growth of the uterus but normal maturation was not achieved, and the uterus remained hypoplastic in half the girls. Recently published data by Snajderova *et al.* (2003) confirmed this observation.

It was shown that GH treatment itself had a positive effect on uterine size in young TS girls in comparison to untreated girls (Sampaolo *et al.*, 2003), whereas Snajderova *et al.* (2003) found no effect of GH therapy on uterine size. Uterine sizes in our women after GH therapy correspond well to published data of TS women without GH therapy.

There is no doubt that adequate estrogen substitution is important to achieve a normal uterine size. However, the evidence showing that estrogen replacement for the induction of puberty limits further gains in height has postponed the introduction of estrogens in Turner girls on GH therapy (Cacciari and Mazzanti, 1999; Chernausek *et al.*, 2000). With respect to uterine size, it remains speculative whether an earlier low-dose estrogen substitution might induce a normal size in all women. On the other hand, there is also increasing evidence that even pre-pubertal ovaries of healthy girls are producing small amounts of estradiol. By using an ultrasensitive estradiol assay, it was shown that TS girls between the ages of 5 and 12 years have significantly lower levels of estradiol than normal pre-pubertal girls (Wilson *et al.*, 2003). Therefore, it could be speculated that a physiological secretion of small amounts of estradiol even through infancy is necessary for the development of a normal uterine size.

It might also be of interest to analyse the used estrogens for pubertal induction, since it has been shown that normal uterine maturation occurred in only 50% of girls who received a treatment protocol using low-dose oral ethinyl estradiol (Paterson *et al.*, 2002). Additionally, positive effects on uterine development in relation to a higher daily dose of estrogen during the post-menarcheal period were reported (Snajderova *et al.*, 2003).

Structural anomalies of the uterus in some TS women may also prevent a normal uterine development. This is supported by data showing that uterine dimensions in TS girls with spontaneous puberty increased with advancing puberty but remained lower than values in a control group (Mazzanti *et al.*, 1997; Haber and Ranke, 1999). It is also interesting that in relation to poor uterine development, breast development was also impaired in 18.6% of our women with karyotype 45,X and stopped at Tanner stage 3, despite appropriate estrogen replacement therapy. Since onset and course of breast development and uterine growth are closely correlated

(Bridges *et al.* 1996), our results are in agreement with the hypothesis of a disturbance affecting the uterine anlage as well as the mammary anlage.

In conclusion, our data show that only TS women with karyotype 45,X/46,XX had normal uterine sizes, whereas 26% of the TS women with karyotype 45,X had a reduced uterine length (< -2 SDS), and 18% a reduced volume (< -2 SDS). However, the data also show that the majority of young adult TS women with different karyotypes can achieve normal adult uterine dimensions with the use of adequate HRT.

Acknowledgements

The participating investigators are as follows: H.I.Akkurt (Hamburg), W.Andler (Datteln), M.Becker (Wiesbaden), H.J.Böhles (Frankfurt), J.Brämswig (Münster), O.Butenandt (München), M.Bettendorf (Heidelberg), H.G.Doerr (Erlangen), J.H.H.Ehrich (Hannover), B.Erxleben (Potsdam), B.Griefahn (Greifswald), A.Grueters (Berlin), K.Hartmann (Frankfurt), B.P.Hauffa (Essen), F.Haverkamp (Bonn), W.Hecker (Stuttgart), U.Heinrich (Heidelberg), G.K.Hinkel (Dresden), O.Hiort (Lübeck), R.Holl (Giessen), J.Homoki (Ulm), G Horneff (Halle), U.Irle (Bremen), R.Johs (Braunschweig), C.Jourdan (Herford), E.Keller (Leipzig), J.Kerstan (Hildesheim), E.Korsch (Köln), J.Kreuder (Giessen), H.P.Krohn (Wilhelmshaven), F.Krull (Aurich), D.Kunze (München), M.Lakomek (Göttingen), D.Lenz (Bremerhaven), U.Leuthold (Siegen), M.Mix (Rostock), K.Mohnike (Magdeburg), M.Morlot (Hannover), R.Mühlenberg (Krefeld), H.Müller (Oldenburg), C.J.Partsch (Kiel), M.Pavlovic (Freiburg), K.Rager (Bad Mergentheim), M.B.Ranke (Tübingen), W.Rauh (Trier), H.J.Rickers (Osnabrück), B.Schenk (Schwerin), D.Schnabel (Berlin), E.Schönau (Köln), H.P.Schwarz (München), G.Simic-Schleicher (Bremen), W.G.Sippell (Kiel), N.Stahnke (Hamburg), M.Teufel (Böblingen), W.Tillmann (Minden), B.Tittel (Dresden), U.Tuschy (Erfurt), K.P.Ullrich (Gotha), W.von Petrikowsky (Freiburg), H.P.Weber (Lüdenscheid), K.Wesseler (Detmold), R.P.Willig (Hamburg), J.Wintgens (Mönchengladbach) and M.Witsch (Mannheim). The study was supported by a grant from Pfizer Corporation, Germany.

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Submitted on June 1, 2004; resubmitted on December 19, 2004; accepted on January 7, 2005