RESEARCH ARTICLE

Association between Karyotype, Puberty Stage, and Volume of Reproductive Organs in Turner Syndrome: The First Transrectal Sonography Study in Indonesia

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Abstract

Background: Turner syndrome is characterized by the absence of part or the entire X chromosome in a woman, resulting in short stature, gonadal dysgenesis, and congenital anomaly. Based on karyotype, Turner syndrome is categorized as classical (45XO) and mosaic. The aim of this study is to evaluate the relationship of the karyotype with the puberty stage, volume of the uterus, and volume of the ovary in adolescents with Turner syndrome who underwent estrogen therapy.

Methods: A cross-sectional study was done at the Cipto Mangunkusumo National Hospital since July to December 2018. Pubertal staging was based on Tanner stage. Uterine and ovary volume were measured using transrectal sonography.

Results: Of 21 study subjects, 8 had classical karyotype and 13 had mosaic karyotype in 12–21-year-old subjects. There was an association between karyotype and Tanner M stage (mammae stage) ($p = 0.035$). Breasts development in Turner syndrome with mosaic karyotype showed better development compared with classic karyotype. No association was found between karyotype and the volume of the uterus and ovaries.

Conclusions: Karyotype is associated with Tanner mammae stage but not with uterine and ovary volume in adolescents with Turner syndrome who underwent estrogen therapy.

Keywords: Karyotype, Ovary, Puberty, Transrectal sonography**,** Turner syndrome, Uterus.

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Background

Turner syndrome is a chromosomal abnormality that happens randomly instead of genetically inherited (unless when there is a partial deletion in X chromosome). It is also known as monosomy X syndrome or Ullrich syndrome.[1] About 50% of all Turner syndrome populations are categorized as classical Turner syndrome (45XO). Twenty-five percent of Turner syndrome population is having partial deletion of an X chromosome, and 20% are categorized as mosaic Turner syndrome.[2] The prevalence of Turner syndrome was one out of 2,000–2,500 baby girl births.[3] Female with Turner syndrome might have various characteristics such as short stature, delayed puberty, and ovary insufficiency.[4]

Ovarian failure is commonly found in Turner syndrome. Some of the Turner syndrome patients might still have normal, functional gonads, while in some other, ovaries are often replaced by ovarian stroma. In the latter condition, sufficient estrogen will not be produced. Some consequences of inadequate estrogen production in Turner syndrome were delayed puberty and reproductive organ immaturity.[5] Besides abnormal ovary shape, fetus with Turner syndrome also shows a faster oocyte depletion compared with normal fetus.[6] Eventually, it will cause infantilism and infertility, which usually become the main concerns for patients with Turner syndrome and will affect their confidence and quality of life.[7,8]

Estrogen hormone therapy has become a standard treatment to tackle the consequences of ovarian failure in patients with Turner syndrome. Secondary sex characteristics will show up and menarche will start at Turner syndrome patients who undergo this therapy. It is known from previous studies that estrogen hormone will affect the size and the volume of reproductive organs both in healthy girls and in the girls with Turner syndrome.[9,10] A prospective ultrasonographic (USG) study for 3 years in the Royal Children's Hospital in Australia by McDonnell et al. on 18 girls with Turner syndrome who received estrogen and growth hormone therapy during infancy and adolescent years, showed that normal uterine size can be reached when the exact amount of estrogen in the body can be maintained during puberty.[11] Previous study showed that the size of uterus and ovaries can be used to predict the fertility in healthy women. One study stated that the volume of ovary is correlated with the number of non-growing follicle (NGF) left, thus, the volume of ovary can be used

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to predict the number of the NGF.[12] Another study performed in an in vitro fertilization (IVF) clinic in Boston, measuring the length of the uterus in women with normal uterine anatomy, stated that women with extremely long uterine length were less likely to give live birth, and women with < 6 cm uterine length were more likely to get spontaneous abortion.[13] Rarely, Turner syndrome patients might be able to have a normal term pregnancy and give birth. A study in France found out that 5.6% (27 of 450 samples) women with Turner syndrome had spontaneous pregnancy. However, there are many complications that might happen along the way during pregnancy, such as miscarriage which can be caused by either diminished endometrial receptivity or chromosomal abnormality in the children.[14] Thus, it is possible that the size of the reproductive organs in Turner syndrome patients can be used to determine whether it is possible for them to get pregnant in the future and whether IVF through oocyte donation or ovarian cryopreservation could become an option if they want to have children in the future. The size of the reproductive organs can also be used to monitor the effectivity of the estrogen hormone therapy as sex hormones influence their size.

Ultrasonography is a widely available diagnostic tool which is used to examine internal genital condition including the uterus, ovary, and adnexa. Routes of examination in USG include transvaginal, transabdominal, and transrectal. Transvaginal USG has several benefits compared to transabdominal, such as a clearer image production, the target organ can be located at focal distance, and the probe can be put within the reach of target organ area. However, there are several limitations too when using transvaginal USG, for example, it cannot be done when there is vaginal agenesis, on women with intact hymen (on virgins), and when there is a possibility of infection. Transrectal USG is as effective as transvaginal USG to obtain internal genitalia structure, as its probe is still located within the reach of target organ area, and considered to be more effective to visualize the reproductive organs compared to transabdominal USG.[15,16]

The purpose of this study was to examine the relationship between karyotype, Tanner puberty stage, and also the volume of the reproductive organs (uterus and ovaries) using transrectal sonography. Tanner puberty stage and volume of reproductive organs can be used to predict the prognosis of adolescents with Turner syndrome, and transrectal USG could give a clearer, more precise result of the reproductive organs measurement.

Methods

Subjects

Subjects were obtained from the Pediatric Endocrinology Polyclinic and Obstetrics–Gynecology Endocrine Outpatient at Cipto Mangunkusumo National Hospital. The subjects were the members of the Turner Syndrome Society at Jakarta, Bogor, Depok, Tangerang, Bekasi, West Java, and Bandar Lampung from July to December 2018. Twenty-one subjects with complete data, who met the inclusion criteria (adolescents diagnosed with Turner syndrome, age range within 12–21 years, and underwent estrogen hormone therapy for at least one month) and did not meet the exclusion criteria (had history of malignancy in adrenal glands, uterine, and ovarium, had chronic/terminal disease such as cyanotic congenital heart disease and tuberculosis in reproductive organs, or if the parents refused to participate) were included in this study. Transrectal USG examination was done in the Obstetrics-Gynecology Endocrine Outpatient Clinic, Cipto Mangunkusumo Hospital. All of the subjects were female. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia.

Definition

Turner syndrome is defined as a syndrome or a group of symptoms in a woman who consists of short stature, gonadal dysgenesis, and major and minor congenital anomaly caused by an abnormality in all or parts of an X chromosome.[1,2] Karyotype is the number and visual appearance of the chromosomes in the cell nuclei.[1] Puberty stage is the transition period between childhood and adulthood which is affected by various factors, marked by physical (secondary sex characteristics) and psychological changes that happen because of the sequential and regular change in endocrine activity.[17] Delayed puberty in girls happens when secondary sex characteristics does not happen until 13 years old.[17] Puberty stage is classified based on Tanner scale and in this study was done by comparing secondary sex characteristics in patients with tables and pictures in Tanner scale.[17] Hormone therapy is estrogen hormone replacement therapy given to the Turner syndrome patients.[17] Uterine and ovarium transrectal USG is a radiologic imaging diagnostic examination done rectally by an experienced and trained obstetrics–gynecologist specialist, using Alpinion E-Cube 7 Diamond USG machine in this study.[5,15] Uterine and left and right ovary volume were determined by measuring maximal longitudinal, anteroposterior, and transversal diameter (in centimeter): uterine volume formula (mL) = longitudinal diameter × transversal diameter × anteroposterior diameter × 0.5233.[5,15]

Statistical Analysis

This was a cross-sectional study which was done to evaluate the relationship between karyotype, puberty stage, and volume of the reproductive organs (ovaries and uterus) in female adolescents with Turner syndrome who received estrogen hormone therapy. Statistical analysis which was used to determine the relationship between karyotype, puberty stage, and reproductive organs Mann–Whitney test. Multivariate analysis using linear regression test was done to evaluate the relationship between karyotype, uterine size and volume, and left and right ovary volume based on the duration of hormone therapy. The data were analyzed using SPSS 23.0 version and considered as significant if p value < 0.05.

Results

The subjects consisted of 13 mosaic karyotype and 8 classical karyotype. The karyotype distribution of the subjects was described in Table 1.

Sixty-one percent of the subjects in this study consisted of mosaic karyotype and 38% of the subjects consisted of classical karyotype, as shown in Table 2.

All of the subjects received estrogen hormone therapy. The mean of the duration of estrogen hormone therapy was 2.1 (SD = 2.26) years for classical karyotype and 3.5 (SD = 2.65) years for mosaic karyotype (Table 3).

The mean age when the diagnosis was made for classical karyotype group was 11.25 (SD = 5.18) years while the mean age for mosaic karyotype group was 12.31 (SD = 4.19) years (Table 4).

Estrogen hormone used was either natural or synthetic estrogen. Spontaneous menstruation only happened in one patient with mosaic karyotype. The rest of the subjects started to have menarche after estrogen hormone therapy introduction

Table 1: Karyotype distribution

Karyotype	*n*	*Percentage*	*Total (%)*
Classical			
45XO	8	38	38
Mosaics			
45XO/46XX	6	28	62
45XO/46Xi(X)(q10)	4	19	
46Xi(X)(q10)/46XX	1	5	
45XO/46XY	1	5	
45XO/46XY/47XYY	1	5	

Table 2: Turner syndrome characteristics

Characteristics	*Classical (n = 8) (mean ± SD)*	*Mosaic (n = 13) (mean ± SD)*
Age (years)	15.9 (2.53)	18.2 (3.11)
Age when diagnosed (years)	11.3 (5.18)	12.3 (4.19)
Age of estrogen hormone therapy initiation (years)	14.0 (0.76)	14.3 (1.88)
Estrogen hormone therapy duration (years)	2.1 (2.26)	3.5 (2.65)
Mother's age at conception (years)	25.9 (4.39)	26.0 (3.85)
Father's age at conception (years)	30.5 (4.11)	28.2 (3.31)
Birth weight (gm)	2,720 (240)	2,750 (230)
Birth length (cm)	47.1 (1.46)	47.2 (1.64)

Table 3: Age when diagnosed with turner syndrome

	Karyotype	
	Classical (n = 8)	*Mosaic (n = 13)*
Mean age, years (SD)	11.25 (5.18)	12.31 (4.19)
Range	0–15	5–17
Age group		
Infant (0–2 years)	1 (12.5%)	0 (0.0%)
Children (> 2–12 years)	1 (12.5%)	5 (38.5%)
Adolescent (> 12–18 years)	6 (75%)	8 (61.5%)

Table 4: Types of estrogen hormone therapy and menstruation

Karyotype	*Classical n (%)*	*Mosaic n (%)*
Natural estrogen hormone	6 (29%)	7 (33%)
Synthetic estrogen hormone	2 (9%)	6 (29%)
Spontaneous menstruation	0 (0%)	1 (4%)

Synthetic estrogen: ethinyl estradiol–progestin combination; natural estrogen: estradiol valerat only and estradiol valerat–progestin combination

except for one subject with classical karyotype who had Müllerian dysgenesis (Table 5).

In this study, mosaic karyotype showed better breasts development compared with classical karyotype when examined using Tanner puberty stage. The result of Mann–Whitney analysis showed an association between karyotype and Tanner mammae puberty stage ($p = 0.035$). Mosaic karyotype group showed better breast growth and development compared with classical karyotype as shown in Table 6.

Table 5: Association between karyotype and puberty stage

	Karyotype		
Tanner puberty stage	*Classical (n = 8)*	*Mosaic (n = 13)*	*p-value*[a]
Mammae stage			
Median (range)	2.5 (1–4)	3.0 (1–4)	0.039
Pubic hair stage			
Median (range)	2.5 (1–4)	3.0 (1–5)	0.383

[a]With Mann–Whitney test

Table 6: Association between karyotype, uterine volume and left and right ovary volume

	Karyotype		
	Classical (n = 8)	*Mosaics (n = 13)*	*p-value*[a]
Uterine volume (mL):			
Mean ± SD	6.79 ± 6.43	7.91 ± 6.64	0.426
Median	5.14	5.30	
Range	0[b] – 17.7	1.97 – 21.7	
Ovary volume dextra (mL):			
Mean ± SD	0.47 ± 0.87	0.36 ± 0.41	0.689
Median	0.21	0.24	
Range	0[b] – 2.60	0[c] – 1.28	
Ovary volume sinistra (mL):			
Mean ± SD	0.71 ± 1.15	0.28 ± 0.32	0.663
Median	0.22	0.15	
Range	0[b] – 3.30	0[c] – 1.16	

[a]With Mann–Whitney *U* test; [b]Mullerian dysgenesis in two subjects; [c]Bilateral gonadectomy history in two subjects with mosaics karyotypes in Y chromosome

In this study, no association was found between karyotype and uterine mean volume ($p = 0.426$) and right and left ovary volume ($p = 0.586$, 0.663) as shown in Table 5. However, we can see from the data that the size of the uterus in mosaic karyotype is bigger compared to the classical karyotype as in classical karyotype, the formation of the uterus can be compromised. For example, the finding of Müllerian dysgenesis in two samples with classical karyotype in our study. As for the ovaries, the mean size of both left and right ovaries in classical karyotype is bigger compared to the mean size of the mosaic karyotype. These findings might be a part of clinical consideration when determining the prognosis.

To evaluate the association between karyotype, uterine size and volume, and right and left ovary volume in Turner syndrome based on the duration of estrogen hormone therapy, a linear regression test was done. The result of the linear regression showed no significant correlation ($p > 0.05$) between karyotype and uterine size, uterine volume, and right and left ovary volume, as mentioned at Figures 1 to 4.

Discussion

There were 21 subjects in this study, with the mean age 15.87 years old for classical Turner syndrome group (SD = 2.53) and 18.15 years old for mosaic Turner syndrome group (SD = 3.10). Turner syndrome

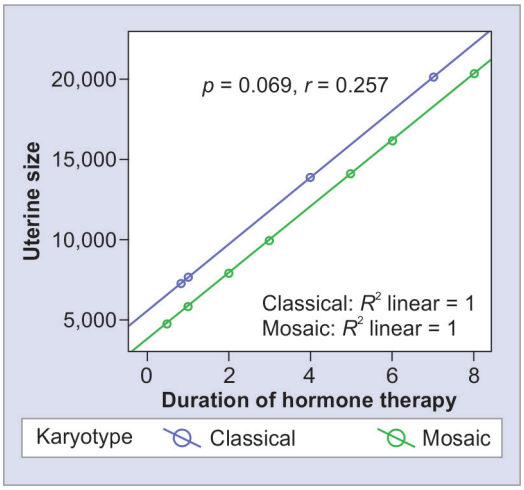

Fig. 1: Association between karyotype and uterine size based on estrogen hormone therapy duration. Linear regression analysis

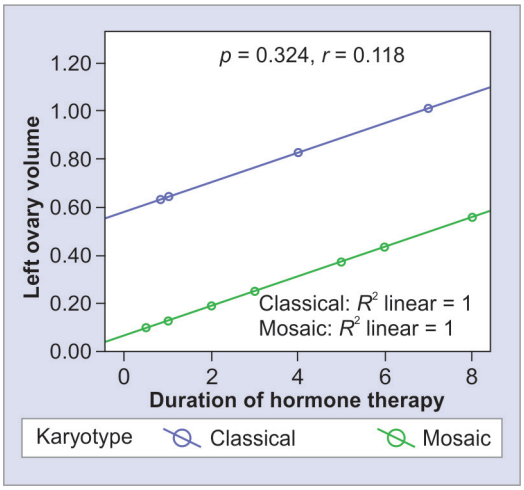

Fig. 3: Association between karyotype and right ovary volume based on estrogen hormone therapy duration. Linear regression analysis

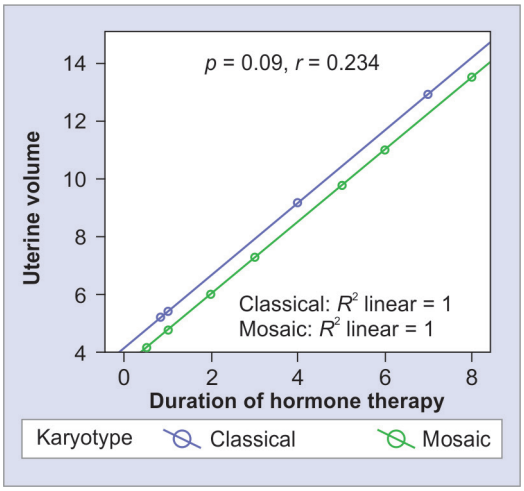

Fig. 2: Association between karyotype and uterine volume based on estrogen hormone therapy duration. Linear regression analysis

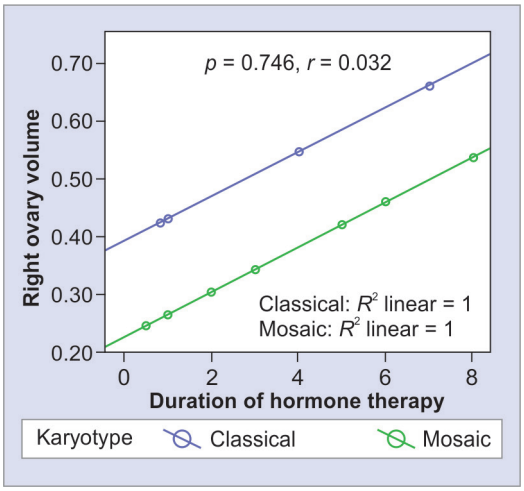

Fig. 4: Association between karyotype and left ovary volume based on estrogen hormone therapy duration. Linear regression analysis

diagnosis was made on various ages, and mostly, the diagnosis was established during adolescence (age group > 12–18 years old). Only 1 out of 21 cases (4.8%) was diagnosed at birth. Some possible USG imaging which might be seen during gestation suggestive of Turner syndrome diagnosis is increased nuchal translucency, cystic hygroma, and left heart obstruction anomaly (especially coarctation aorta).[4] This result might be caused by the low self-consciousness of the parents to do routine antenatal care in Indonesia, although the facilities have been quite well provided. A study done in Jember, East Java, Indonesia, which was done to assess the antenatal care compliance of the third-trimester pregnant women showed a low number (58.2% of total 67 samples).[18] Diagnosis delay will have an impact on the intervention. As a result, the therapy will most likely be delayed.

The mean age of parents when conception happened for both karyotypes was below 35 years and there was no significant difference between classical and mosaic groups ($p > 0.05$). This finding matched the literatures which mentioned that Turner syndrome prevalence was not affected by mother's and father's age at conception.[1,2]

There was no significant difference between both the mean of birth weight and length and karyotypes ($p > 0.05$). In this study, birth weight mean in the classical karyotype was 2.720 gm (SD = 240) and 2.750 gm (SD = 230) in the mosaic karyotype, while birth length mean on classical karyotype and mosaic karyotype was 47.1 cm (SD = 1.46) and 47.2 cm (SD = 1.64), respectively. In Turner syndrome, delayed development, which can be seen in the height, weight, and body mass index measurement, happened since intrauterine phase and continued during childhood, followed by growth spurt failure that happened during puberty.[4,17,19]

Mosaic karyotype made up 62% of karyotype distribution of the subjects in this study and the rest 38% of the subjects consisted of classical karyotype. Previous study prevalence stated that 45XO karyotype made up about 50% of Turner syndrome population, 25% were having partial X chromosome deletion, and 20% were having mosaic karyotype with variety, especially 45XO/46XX, and a small number of Turner syndrome patients carried the XY gene.[2,6]

In this research, the mean age of estrogen hormone therapy initiation was 14.3 (SD = 1.9) years old in the mosaic group and 14.0 (SD = 0.8) in the classical group. As most of the diagnosis in this

study were made on puberty and not earlier, the estrogen hormone therapy could not be started earlier. Based on the recommendation from the Cincinnati International Turner Syndrome Meeting on 2016, fertility therapy had to be given since young age. Mosaic Turner syndrome patients at a young age with functional ovaries can start to be counseled for fertility preservation by oocyte cryopreservation method when they have reached 12 years old.[20] Every subject who were included in this study went through further examination with obstetrics–gynecologist endocrine consultant to examine whether there was a possibility of doing fertility preservation.

Significant difference was found between karyotype and Tanner puberty stage M (*mammae*) ($p = 0.039$). Mosaic karyotype group showed better breast growth and development compared to the classical karyotype group. This finding matched a study done by Wu and Li on 124 classical and mosaic Turner syndrome patients who came to pediatric polyclinic at the Capital Institute of Beijing. Secondary sexual characteristic growths were found to be better in mosaic karyotype.[21] Estrogen hormone plays an important role in breasts development. Low dose of estrogen hormone therapy, when given at appropriate age for children with Turner syndrome, will be helpful to make the breasts grow normally although the normal growth of breasts will be reached at the age 2 years later compared to normal girls.[22]

Pubic hair puberty stage (*p* stage) did not have a significant difference between classical and mosaic karyotypes. This result was similar with a study done by Bannink et al. on 56 children with Turner syndrome prospectively. Pubic hair growth in Turner syndrome patients was the same with normal women, although it was a bit late. In Turner syndrome, androgen hormone disturbance only happened in the ovary while androgen hormone remains normal at the adrenal gland; thus, the adrenarche/pubarche in Turner syndrome is the same with normal women.[22]

No significant difference was found between karyotype and mean uterine, right and left ovary volume ($p = 0.426$, 0.689, 0.663, respectively). We also found that the mean of uterine length and volume in patients with classical karyotype were smaller than the findings on the mosaic karyotype, similar with the finding in a cross-sectional study done by Haber and Ranke. In general, the volume of uterus and ovaries of the Turner syndrome patients in our study were smaller compared to the result obtained from the previous study by Haber and Ranke, where the volume of the reproductive organs were imagined through transabdominal USG in 93 Turner syndrome patients and in 190 normal, healthy girls as control group. The age range of the samples was 12 days old until 17.85 years old. A standard reference for uterine and ovary volume of the Turner syndrome patients had been established through that study, where the volume of the uterus in post-pubertal patients with Turner syndrome was 8.3 ± 0.4, smaller compared to the volume of the uterus in the control group, which is 15.9 ± 9.7. Meanwhile, the volume of the ovaries in post-pubertal patients with Turner syndrome was 1.4 ± 0.9, and the volume of the ovaries in the control group was 5 ± 2.4. In that study, a significant relationship was found between karyotype and visualization of ovaries. Mean uterine volume of the subjects with Turner syndrome and control group was also found to have a significant difference.[5]

The result of this study was consistent with the finding in the previous cross-sectional study done by Elsedfy et al. at Kairo on 40 Turner syndrome patients aged 9.71–26.32 years, using transabdominal USG. The size of uterine in that study was not affected by karyotype ($p = 0.40$). Elsedfy et al. also analyzed the relationship between uterine size and the type of therapy, and the study showed that no relationship was found between those variables.[23] A research done by Liang et al. in 51 children with Turner syndrome compared to 20 healthy girls also stated that the size of uterus in children with 45XO karyotype in Turner syndrome was smaller compared to the other types besides 45XO ($p < 0.05$).[24]

We also found two subjects with classical karyotype whose uterus and ovaries were hard to identify using transrectal USG. Mullerian dysgenesis was suspected in these patients. This condition was similar with the finding in a study done by Haber and Ranke, which stated that only 41 (44%) of 93 Turner syndrome patients' ovaries can be visualized. In Turner syndrome patients, the shape of the ovary varies a lot, including streak which contains fibrous tissue and gonad with normal shape and function.[5] Gonadoblastoma might happen during infancy. Thus, it was advised for a Turner syndrome patient who carries Y chromosome material at FISH test to do prophylactic gonadectomy.[17,19] A similar situation was found in a study done by McDonnell et al., which stated that 3 of 18 patients with Turner syndrome with Y chromosome had bilateral gonadectomy.[11] In our two subjects with Y chromosome, bilateral gonadectomy was done as a preventive measure for malignancy. Although gonadectomy had been done, we did not exclude the subjects in the analysis so that we can have a further look on the ovary shape variations in Turner syndrome patients. Linear regression analysis was done by examining the role of karyotype on the shape and volume of the uterine and the right and left ovary volume on the patients who underwent estrogen hormone therapy on certain duration showed that there was no significant correlation between them ($p > 0.05$). This finding is similar with the result of the research done by Elsedfy et al. in 40 patients with Turner syndrome, which stated that karyotype did not affect the size of the uterus ($p = 0.40$).[23]

The strength of this study is this was the first study in the world that used transrectal USG to examine the development of the uterus and ovary in adolescents with Turner syndrome while evaluating its correlation with karyotype. Several previous studies regarding reproductive organ volumes in Turner syndrome had been done using transabdominal USG as mentioned above, but none of those studies had ever used transrectal USG as the imaging method. In our study, transrectal USG examination was done by an experienced obstetrics–gynecology endocrine fertility consultant. The image produced using transrectal USG is supposed to be clearer and more precise compared to using transabdominal USG, thus, the result of the study was deemed to be more accurate compared to previous studies that only used transabdominal USG to determine the size of the reproductive organs.

The limitation of this study is we cannot present the data of the control group, which is supposed to be the volume of the reproductive organs in normal girls examined using transrectal USG.

Conclusions

- In adolescents with Turner syndrome who underwent estrogen hormone therapy, an association was found between karyotype and Tanner mammae puberty stage. Mosaic karyotype showed a better mammae growth and development based on Tanner stage compared to the classical karyotype.
- There was no association between karyotype and the volume of both uterus and ovaries in Turner syndrome patients with the duration of estrogen hormone therapy.

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Availability of Data and Material

The data are available in the corresponding author upon request.

Authors' Contribution

NN and ABP designed the study, created the content, and did the literature research. NN, HG, and KS did the data collection, data analysis and statistical analysis. NN was the major contributor in writing the whole manuscript. All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate

Inform assent and inform consent regarding the purpose of the study, the procedures, and the possible uncomfortableness which might be felt by the subject upon examination were explained verbally to the subject and the parents until they understand thoroughly before any procedure was done. Afterwards, the subject and the parents needed to sign the written informed consent form, stating their agreement whether they agree to participate in this study. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia, July 30, 2018, and had been conformed to the ethical guidelines of the Declaration of Helsinki. The patients were anonymized and de-identified before analysis. Reference number: 0767/UN2.F1/ETIK/2018.

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