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RESEARCH ARTICLE

# Chromosomal abnormalities in primary and secondary amenorrhea

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## ABSTRACT

**Background:** Menstruation is an important physiological function of the female reproductive system. The absence of menstruation is called amenorrhea. Many genetic and nongenetic causes are responsible for primary or secondary amenorrhea. This study aimed to determine the types chromosomal abnormalities among patients with primary or secondary amenorrhea.

**Methods:** It was a cross-sectional study conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, from September 2019 to August 2021. A total of 115 women who had come for a karyotype test with complaints of pathological amenorrhea were purposively selected. One hundred five cases presented with primary amenorrhea, and 10 cases presented with secondary amenorrhea. Karyotype analysis in peripheral blood by G-banding was carried out using the standard method.

**Results:** Among the 105 patients with primary amenorrhea, 53.3% of patients had a normal karyotype (46, XX), and 46.6% had chromosomal abnormalities (numerical or structural). Turner syndrome classic, 45, XO (16.2%) and 46, XY DSD (Disorders of Sexual Development) (11.4%) were the two most frequent chromosomal abnormalities found in the patients with primary amenorrhea. Among the 10 cases with secondary amenorrhea, seven patients had normal karyotype, and three patients had chromosomal abnormalities (2 had sex chromosomal abnormalities, and 1 had a structural abnormality of autosome).

**Conclusion:** The study of chromosomal abnormalities will help in the early and accurate diagnosis of the underlying aetiology of primary or secondary amenorrhea. It will also help in the management and proper counselling of cases.

***Keywords:*** *primary amenorrhea, secondary amenorrhea, chromosomal abnormalities, karyotyping.*

## INTRODUCTION

Amenorrhea means the absence of menstruation. Pathological amenorrhea can be subdivided into primary amenorrhea and secondary amenorrhea. Primary amenorrhea is the absence of menstruation by 16 years of age in the presence of typical secondary sexual characteristics or by 14 years of age if the secondary sexual characteristics have not developed.[1] Secondary amenorrhea is the absence of menstruation for three regular cycles or six months.[1] About 2-5% of women of reproductive age are affected by amenorrhea.[2] In South East and Middle Asia, about 1-3% of women suffer from primary amenorrhea, and 3-4% of women suffer from secondary amenorrhea.[2,3,4] Amenorrhea accounts for 20% of female partners of infertile couples.[5] In our country, the inability to bear a child is a big social stigma. It has a harmful impact on women's psychology and their marital life.

Normal menstruation requires a normal reproductive system with normal chromosomal complement 46, XX. Genetic abnormalities, including chromosomal abnormalities, are a major etiological factor for pathological amenorrhea. Genes present in the X chromosome (i.e. DAX1 gene) and in several autosomes (i.e. WNT4 gene, RSPO1 gene in chromosome 1, FOXL2

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## HIGHLIGHTS

1. This study reports that chromosomal abnormalities are important etiological factors of primary and secondary amenorrhea.
2. Detection of specific chromosomal abnormalities in patients with primary and secondary amenorrhea is very important for patient management and counselling.

gene in chromosome 3) are responsible for the development of the normal female reproductive system. Abnormalities of these chromosomes cause agenesis or maldeveloped organs of the female reproductive system (i.e. mixed gonadal dysgenesis, Mullerian agenesis, vaginal atresia etc). These may result in pathological amenorrhea and infertility. The percentage of chromosomal abnormalities reported varies from 15.9% to 63.3% for primary amenorrhea and from 3.8% to 44.4% for secondary amenorrhea in Turkey.[6]

Karyotyping is very important to determine the cause of primary or secondary amenorrhea. Patients with chromosomal abnormalities need proper management and appropriate counselling. The counselling must be relevant to the type of genetic disease and their reproductive risk, like in Turner syndrome, patients need to know about premature ovarian insufficiency and infertility, and patients with Y chromosome need to know about the prophylactic gonadectomy due to the risk of gonadal malignancy. Hence, the study aimed to determine the frequency and types of chromosomal abnormalities among women with primary and secondary amenorrhea for better management and counselling.

## METHODS

### *Patients*

This cross-sectional study was carried out from September 2019 to August 2021 at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The study population was the patients who had come to the cytogenetics laboratory of the Department of Pathology, BSMMU, for karyotyping with complaints of primary or secondary amenorrhea. The sample size was calculated according to the prevalence of the outcome of pathological amenorrhea ($P$=0.08).[7] A total of 115 cases of primary and secondary amenorrhea were included in this study. The inclusion criteria were ages 14 to 45 years who presented with primary or secondary amenorrhea. Patients with drug, medication or surgery-induced amenorrhea were omitted.

### *Karyotyping*

Standard cytogenetic techniques were used for all the cases to detect chromosomal abnormalities. Karyotyping was performed by using peripheral blood samples of selected cases. With all aseptic precautions, 2-3 mL of venous blood was taken in a heparinised syringe. Microculture was done by allowing the blood samples to stand for about 30 minutes to separate, and then an adequate amount of lymphocyte-rich plasma was put into the culture media in a previously prepared culture tube containing 5 mL karyotyping media. Incubation was done at 37°C for three days in a slanting position. Harvesting was done by adding 3-4 drops of coloemid to the culture tube 1 hour before 72 hours of incubation. Again, incubation was done at 37°C for 45 to 60 minutes after mixing gently. Then, the culture tube was centrifuged at 800-1000 rpm for 10 minutes. The supernatant was discarded by pipetting, and the cell button was collected. The cell button was re-suspended in 5 mL of hypotonic solution and kept in a water bath for 10-12 minutes. Five drops of freshly prepared fixative (methanol: acetic acid= 3:1) were added to the tube, and centrifugation was done at 800 rpm for 5 minutes. The supernatant was discarded, and 3 mL of fixative was added and kept at room temperature for 10 minutes. Again, the tube was centrifuged, and fixative was added after discarding the supernatant. The suspension was maintained for 30 to 60 minutes.

Before slide preparation, the suspension was again centrifuged and freshly prepared fixative was added after discarding the supernatant. 3-4 drops of suspension were dropped over the slide. Quick drying in a flame was done. Then, the slides were kept in hot air woven at 800 to 900 C for 1 hour for ageing. Then, the slides were kept in 0.05% trypsin solution for 15-20 seconds. After rinsing in cold PBS, the slides were flooded with Giemsa staining solution for 15-20 minutes. Again, the rinsing was done in distilled water,

and the slides were air-dried. Two slides were made for each case. Each slide was first scanned under low magnification (10X) to locate a good-quality spread. Then chromosomal analysis was done according to the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN, 2013) in at least 100 well-spread and well-banded G-banded metaphase for each patient and analysed for aneuploidy and other structural abnormalities by using the GTG-banding technique with the help of Cytovision software version 7.5 (Leica biosystem) at 400 to 550 band resolutions.

### *Statistical analysis*

Statistical analyses were carried out using the Microsoft Excel 2010 software. Frequencies and percentages of the genotypes were presented.

### *Ethical implication*

Every ethical issue regarding the study was discussed with the patients and informed written consent was obtained in the performed data collection form before the procedure. Anonymity of the data was maintained. The study procedure also conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

## RESULTS

In this study, 105 patients had primary amenorrhea, and ten patients had secondary amenorrhea. The age range of 91.4% of cases of primary amenorrhea was 14 to 25 years (mean age, 18 years), and 8.6% of cases were 26 to 35 years. 90% of cases of secondary amenorrhea were 14 to 25 years, and 10% of cases were 26 to 35 years. Among 105 patients with primary amenorrhea, 53.3% had normal karyotype (46, XX), and 46.7% of patients had structural and numerical chromosomal abnormalities **(TABLE 1)**. Most common (16.2%) chromosomal abnormality was 45, XO (Turner syndrome) **(FIGURE 1)**. The second, third and fourth frequent group of chromosomal abnormality were 46, XY **(FIGURE 2),** 46, Xiso(Xq) **(FIGURE 3)**, and 45, XO/46, Xiso(Xq) **(FIGURE 4)**.

**TABLE 1 Karyotype of the study cases with primary amenorrhea (n=105)**

| Karyotype | Frequency | Percent |
|---|---|---|
| 46, XX | 56 | 53.3 |
| 45, XO | 17 | 16.2 |
| 46, XY | 12 | 11.4 |
| 46, Xiso (Xq) | 5 | 4.8 |
| 45, XO/46, Xiso (Xq) | 4 | 3.8 |
| 45, XO/46, XY | 3 | 2.9 |
| 46, XX,der(15)t(?Y;15) | 2 | 1.9 |
| [a]Others | 6 | 5.4 |

[a]46, Xdel(X)(q21-q28); 46, Xdup(X), (q21-q26); 46, XY,inv(7)(p15;q22); 46, XX,inv(9)(p12;q13); 46, XX,inv(9)(p12;q22); 46, XX,t(14/15) one each

**TABLE 2 Karyotype of the study cases with secondary amenorrhea (n=10)**

| Karyotype | Frequency | Percent |
|---|---|---|
| 46, XX | 7 | 70.0 |
| 45, XO/46, XY | 1 | 10.0 |
| 46, Xiso (Xq) | 1 | 10.0 |
| 46, XXdup (9) (q12-q13) | 1 | 10.0 |

Among ten patients with secondary amenorrhea, seven patients had normal karyotype (46, XX). The other three patients had 45, XO/46, XY (mixed gonadal dysgenesis), 46, Xiso(Xq) (Turner syndrome) and 46, XX, dup (9)(q12-13) (Partial trisomy of chromosome 9) respectively **(TABLE 2)**.

## DISCUSSION

In the present study, the mean age of primary amenorrhea was 18 years. The result is consistent with another study where the mean age was 21.[8] The mean age of secondary amenorrhea in this study was 22 years, which is consistent with another study where the mean age was 26.5 years.[9]

Several studies have been carried out to determine the frequency of sex chromosome abnormalities among patients with primary and secondary amenorrhea and found that chromosomal abnormalities are present in 46% to 62% of patients with primary amenorrhea.[2] The chromosomal abnormalities may include X aneuploidy, male karyotype, or structural X chromosome abnormalities (isochromosome, duplications, deletions, translocations or inversion of X chromosome).[5] The prevalence of chromosomal abnormalities in secondary amenorrhea varies from 3.8 to 44% in different parts of the world.[9, 10] In this study, among 105 patients with primary amenorrhea, 53.4% patients had normal karyotype 46, XX and 46.6% patients had chromosomal abnormalities. Several other studies found chromosomal abnormalities ranging from 13.77% to 38.9%.[3, 6, 7, 11]

FIGURE 1 Karyotype of 45, XO Turner syndrome classic

FIGURE 2 A karyotype of 46, XY DSD

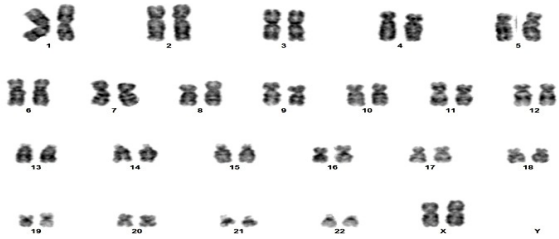

FIGURE 3 Karyotype of 46, Xiso (Xq) Turner syndrome

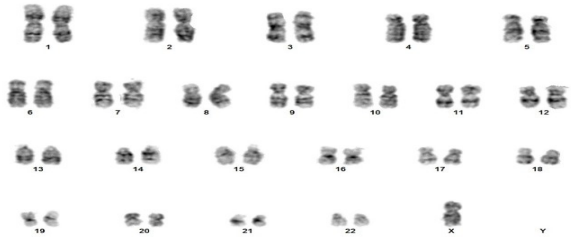

FIGURE 4 A karyotype of 45, XO/46, Xiso(Xq) mosaic Turner syndrome

Among ten patients with secondary amenorrhea, seven cases had normal karyotype 46, XX and three had chromosomal abnormalities. The estimated frequencies of chromosomal abnormalities in secondary amenorrhea described by other studies were 0.5% and 21.8%.[6, 12] This variation might be due to the variation in the sample size and different karyotyping techniques. Besides nongenetic causes, women with normal karyotypes (46, XX) may have primary or secondary amenorrhea due to abnormalities of autosomal genes. Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome may be associated with primary or secondary amenorrhea in women with normal karyotypes. Loss-of-function mutation in early Mullerian duct patterning genes such as LIM1, WNT4, and WNT9B can cause MRKH syndrome. Deletion of 22q11 is also associated with this syndrome. Most cases of MRKH have no known aetiology and could be genetic or environmental. WNT4, RSPO1 and FOXL2 are essential genes for normal ovarian development and maintenance. Mutation of this gene is also responsible for ovarian insufficiency and causes amenorrhea. The absence of the uterus is pathognomonic of the WNT4 (1p31-p35) gene defect.[13] In this study, the majority of the study cases had normal karyotype 46, XX. Amenorrhea with this karyotype is possibly due to any of the causes like Mullerian agenesis (MRKH syndrome), primary dysfunction of the ovary, disruption of hypothalamic or pituitary function, anatomic disease affecting the hypothalamic-pituitary-gonadal axis and pathology of other endocrine glands.[14]

The most frequent group of chromosomal abnormalities in primary amenorrhea found in this study was Turner syndrome classic (45, X). It is the most common cause of primary amenorrhea. About 90% of Turner syndrome patients experience primary amenorrhea.[15] In the present study, it constituted 16.2% of the cases of primary amenorrhea. Similar findings were also reported by some other studies in different countries.[5, 7, 11] 45,XO karyotype was absent in patients with secondary amenorrhea in this study.

In this study, the second most common karyotype in primary amenorrhea was 46, XY (11.4%). Another study obtained a similar result.[5] According to other studies, this karyotype was the third most common abnormal karyotype.[5, 16] This female sex reversed 46, XY karyotype is associated with Swyer syndrome, is a unique condition with female phenotype but had 46, XY karyotype. This is due to mutation or deletion of the SRY gene on the Y chromosome at Yp11.3. This results in testicular dysgenesis and does not produce

testosterone or anti-Mullerian hormone. Thus, the Mullerian duct persists, and the patient may have a rudimentary uterus and vagina. As for normal ovarian development and function, the presence of both XX chromosomes is important, so in this condition of pure gonadal dysgenesis, females have streak gonads and are presented with amenorrhea.[17]

The third commonest abnormal karyotype of this study was 46, Xiso (Xq), a variant of Turner syndrome, which constituted 4.8% of cases in primary amenorrhea. This finding was comparable to the results obtained by two other studies.[5, 6] Among 10 cases of secondary amenorrhea, this karyotype was found only in 1(10%) patient. The results of another study were comparable to this finding.[10] This variant of Turner syndrome involves partial deletion of the second X chromosome and contains two copies of q-arm. Genes involved in gonadal function are located in the proximal part of Xp and the distal part of the Xq. In the absence of Xp, patients with this karyotype have gonadal dysgenesis and present with amenorrhea.[6]

This study's fourth frequent group of chromosomal abnormality was 45, XO/46, Xiso(Xq). This mosaic Turner syndrome with structural abnormality constituted 3.8% of cases in primary amenorrhea. A similar finding was observed in several other studies.[6, 11] This karyotype was absent in patients with secondary amenorrhea in this study. This form of mosaic Turner is due to initial X chromosome breakage, fusion, and eventually unipolar segregation. The complete or partial loss of the X chromosome may lead to streak gonads and cause amenorrhea. This is also associated with gonadal dysgenesis.[4]

The fifth predominant abnormal karyotype was 45, XO/46, XY, suggesting mixed gonadal dysgenesis. It comprised 2.9% of the total study cases in primary amenorrhea. Gonadal dysgenesis refers to various diseases in which the development of the indifferent embryonic gonads to differentiated gonads is inhibited. Mutation in the SRY gene is considered one of the etiologies.[17] The result in this study was similar to the results obtained by the other two studies.[5, 7] In secondary amenorrhea 45, XO/46, XY karyotype was present in 10% of the study population. Another study found a similar result.[10] This Karyotype reflect Turner syndrome with XY cell line. SRY gene mutation may be involved in this condition. These patients may have typical Turner syndrome phenotype, normal male appearance, male pseudo hermaphroditism and mixed gonadal dysgenesis.

The other karyotypes with structural abnormalities found in this study were duplications, deletions, or inversion of the X chromosome. In our study, 46, XX, dup (9) (q12-13) karyotype was found in 10% of patients with secondary amenorrhea. Premature ovarian failure may occur secondary to structural defects of the X chromosome. The deleterious effects on ovarian development and function result from these structural abnormalities involving the long arm of the X chromosome between Xq13 and Xq26. This is a critical region for normal ovarian function. POF1 and POF2 genes are located at Xq21.3-27 and Xq13.3-Xq21.1, respectively, essential for normal ovarian development and functions. Sensitive structural changes of these regions result in pachytene atresia or apoptosis of oocytes and cause amenorrhea.[9] 46, XX, der(15)t(?Y;15), an unbalanced chromosomal translocation causes the progression of oogenesis without an arrest in meiosis and produces abnormal oocytes with unbalanced chromosomal constituents with abnormal ovarian functions. However, these types of structural abnormalities need molecular tests (i.e. FISH) for confirmation.[18]

The majority of the cases of primary amenorrhea and all cases of secondary amenorrhea with 46, XX karyotype had normal height, normal level of reproductive and thyroid hormones with well-developed breast, axillary hair, pubic hair and external genitalia. 92.8% of patients with primary amenorrhea with abnormal karyotype had short stature. All the cases of secondary amenorrhea with abnormal karyotypes had normal height. 50.5% cases of primary amenorrhea and 20% cases of secondary amenorrhea with abnormal karyotype (Turner syndrome classic and variants, mosaic Turner syndrome, mixed gonadal dysgenesis, 46, XY DSD and others) had hypoplastic or absent ovaries with low levels of estrogen and raised FSH, LH level. These patients

had poorly developed breasts and axillary and pubic hair but had normal external genitalia. Some of these patients had hypoplastic uterus. Developmental abnormalities of ovaries or uterus are possibly due to the loss of part or an entire X chromosome or the presence of SRY mutated Y chromosome, which prevents differentiation of testes and results in gonadal dysgenesis.

This study may reflect the frequency and types of chromosomal abnormalities among women with primary and secondary amenorrhea for their proper management. In this study, fluorescence in situ hybridisation, comparative genomic hybridization, or next-generation sequencing for detecting specific genetic mutations (e.g., microdeletion) were not possible due to limited resources and the unavailability of the facilities.

### *Conclusion*

The results of the study concluded that a significant number of patients with primary and secondary amenorrhea had abnormal karyotypes, and there is a higher prevalence of chromosomal abnormality in primary amenorrhea than in secondary amenorrhea. An early diagnosis by karyotype analysis can help in patient management in a better way with possible therapies and genetic counselling. The counselling should include her reproductive life, the risk of infertility, gonadal malignancy, etc.

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#### Author contributions

*Conception and design:* TBA, SH. *Acquisition, analysis and interpretation of data:* TBA, SH, BPD. *Manuscript drafting and revising it critically:* TBA, JAJ, SH, BPD. *Approval of the final version of the manuscript:* TBA, JAJ, SH, BPD. *Guarantor of accuracy and integrity of the work:* TBA.

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#### Conflict of interest

We do not have any conflict of interest.

#### Ethical approval

The Institutional Review Board of BSMMU gave ethical approval in Memo No. BSMMU/2020/8778, dated 04 October 2020.

#### Data availability statement

We confirm that the data supporting the findings of this study will be shared upon reasonable request.

## REFERENCES

1. Malhotra N, Malhotra J, Saxena R, Bora NM. Jeffcoate's principles of gynaecology. 9th ed. JP Medical Ltd; 2018 Aug 16: 112-116.
2. Al-Jaroudi D, Hijazi A, Bashir M, Heena H, Tashkandi SA. Chromosomal aberrations in women with primary and secondary amenorrhea: A cross-sectional study. J Obstet Gynaecol Res. 2019 Aug;45(8):1497-1505. DOI: https://doi.org/10.1111/jog.14006.
3. Sultana S, Sultana N, Ullah SA, Walida SA, Akter P, F Ahmed. Study of Primary Amenorrhea with aetiology in outdoor patients of Rupganj Upazilla Health Complex. Northern International Medical College Journal. 2018 Jan;9(2):300-303. DOI: http://dx.doi.org/10.3329/nimcj.v9i2.38911
4. Samarakoon L, Sirisena ND, Wettasinghe KT, Kariyawasam KW, Jayasekara RW, Dissanayake VH. Prevalence of chromosomal abnormalities in Sri Lankan women with primary amenorrhea. J Obstet Gynaecol Res. 2013 May;39 (5):991-7. DOI: https://doi.org/10.1111/j.1447-0756.2012.02063.x.
5. Mohajertehran F, Ghodsi K, Hafizi L, Rezaee A. Frequency and the type of chromosomal abnormalities in patients with primary amenorrhea in northeast of iran. Iran J Basic Med Sci. 2013 Apr;16(4):634-9. PMID: 24250941.
6. Demirhan O, Tanrıverdi N, Tunç E, Inandıklıoğlu N, Süleymanova D. Frequency and types of chromosomal abnormalities in Turkish women with amenorrhea. J Pediatr Adolesc Gynecol. 2014 Oct;27(5):274-7. DOI: https://doi.org/10.1016/j.jpag.2013.11.013.
7. Dutta UR, Ponnala R, Pidugu VK, Dalal AB. Chromosomal abnormalities in amenorrhea: a retrospective study and review of 637 patients in South India. Arch Iran Med. 2013 May;16(5):267-70. PMID: 23641739.
8. Koppaka NT, Virulkar SK, Chavan DS, Dalvi RC, Gupta N, Mandava S. Specific Chromosomal Aberrations in Primary Amenorrhoea: Study on 3776 Cases from Indian Population. J Obstet Gynaecol India. 2019 Oct;69(5):457-461. DOI: https://doi.org/10.1007/s13224-019-01209-3.
9. Safai A, Vasei M, Attaranzadeh A, Azad F, Tabibi N. Chromosomal Abnormality in Patients with Secondary Amenorrhea. Arch Iran Med. 2012; 15(4): 232 – 234. PMID:22424042.
10. Rajangam S, Nanjappa L. Cytogenetic studies in amenorrhea. Saudi Med J. 2007 Feb;28(2):187-92. PMID: 17268694.
11. Rajitha V., Rekha G., Nathan SS. A Study of Cytogenetic Pattern in Amenorrhea. International Journal of Anatomy, Radiology and Surgery. 2017 Jul;6(3): AO10-AO15. DOI: https://doi.org/10.7860/IJARS/2017/29103:2295.
12. H. Afshar, R. Najafipour, j. JAnsari, N.Karimi, M. Jalilvand. Cytogenetic analysis in women with primary and secondary amenorrhea in Iran: a retrospective study on 110 patients. Journal of Fundamental and Applied Sciences. 2016 August; 8(2):1173. DOI: https://doi.org/10.4314/jfas.8vi2s.107
13. Roly ZY, Backhouse B, Cutting A, Tan TY, Sinclair AH, Ayers KL, Major AT, Smith CA. The cell biology and molecular genetics of Müllerian duct development. Wiley Interdiscip Rev Dev Biol. 2018 May;7(3):e310. DOI: https://doi.org/10.1002/wdev.310.

14. Klein DA, Poth MA. Amenorrhea: an approach to diagnosis and management. Am Fam Physician. 2013 Jun 1;87(11):781-8. PMID: 23939500.

15. Davenport ML. Approach to the patient with Turner syndrome. J Clin Endocrinol Metab. 2010 Apr;95(4):1487-95. DOI: https://doi.org/10.1210/jc.2009-0926.

16. Motamedirad N, Hosseini S, Ebrahimzadeh-Vesal R, Tootian S, Abbaszadegan MR. Investigation of the Frequency and Type of Chromosomal Abnormalities in Women Patients with Amenorrhea. Reports of Biochemistry & Molecular Biology. 2022 Oct;11(3):450. DOI: https://doi.org/10.52547/rbmb.11.3.450.

17. Samal R, Habeebullah S. Primary amenorrhea: a clinical review. Int J Reprod Contracept Obstet Gynecol 2017; 6:4748-4753. DOI: https://doi.org/10.18203/2320-1770.ijrcog20174981.

18. Chen PY, Yen JH, Cheng CF, Chen PC, Li YS, Li TY, Yeh CN, Fang JS. Prenatal diagnosis of the maternal derivative chromosome der(15)t(Y;15) (q12; p13) in a dizygotic twin pregnancy. Ci Ji Yi Xue Za Zhi. 2016 Oct-Dec;28(4):176-179. DOI: https://doi.org/10.1016/j.tcmj.2016.06.002.