

Clinical and Genetic Features of Women's Cohort with Turner Syndrome from Lviv Region (West Ukraine) for 1997-2017

Kitsera N^{*}, Helner N, Osadchuk Z, Bezkorovaina G and Mikula M

Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine

^{*}Corresponding author: Kitsera N, Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine, Tel: +0380973342609; E-mail: nkitsera@gmail.com

Received Date: March 10, 2018; Accepted Date: April 2, 2018; Published Date: April 17, 2018

Copyright: © 2018 Kitsera N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Turner syndrome (TS) is a chromosomal disorder caused by complete or partial X chromosome monosomy that manifests various clinical features depending on the karyotype and on the genetic background of affected girls. Aim of our study was to assess the clinical and the cytogenetic characteristics in patient with Turner syndrome from Lviv region (West Ukraine) for 1997-2017.

Methods: 135 female patients aged 1 month-49 years from Lviv region were clinical and cytogenetically diagnosed Turner syndrome with conventional G-banding.

Results: The average age at diagnosis of 135 patients was 16.9 ± 5.3 years. From 1997-2017 years the majority of patient with TS have been represented at the age category of 12-18 years, 55 people (40.7%). In 12, 6% girls diagnosis was established in the 1st year of life.

There were 73 cases with monosomy X (54.1%), mosaicism 51 (37.8%), only structural X chromosome abnormalities without mosaic forms was found in 11 patients (8.1%). We observed mosaicism (45,X/46,XX) in 15.6%, mosaicism with polysomy X chromosome (45,X/47,XXX) in 4.4%, 45,X/46,X,i(Xq) in 8.9%, mosaicism 45,X/46,X,r(X) in 3.7% and 7(5.2%) other cases of mosaicism. Among our cohort patients in 113 (84.4%) women clinically diagnosis was suspected. Women with Turner syndrome are more likely to have abnormality of skeletal system in 78 (57.8%) and pathology of endocrine system 57% (77 cases), especially thyroid dysfunction and reproductive system – 51.1% (69 cases).

Conclusion: In Lviv region (West Ukraine) the highest incidence of primary diagnosis of TS is after 12 years old. The largest proportion of patients with TS has a karyotype with monosomy X.

Keywords: Turner syndrome; X chromosome; Phenotype; Age; Ukraine

Introduction

Turner syndrome is a genetic disorder characterized by the (partial) absence or a structural aberration of the second sex chromosome and is associated with a variety of phenotypes with specific physical features and different malformations. Turner syndrome is highly variable and can differ dramatically from one person to another [1-3]. First described by Henry Hubert Turner, American endocrinologist in 1938, in a patient with sexual infantilism, webbed neck, cubitus valgus, and short stature [4]. Turner syndrome is one of the most common chromosomal abnormalities, with an annual incidence since 1:1500 to 1:4000 live born female infants [5-8]. Different authors often indicates frequency 1:2500 [1,9]. So 13 years incidence study of sex chromosome abnormalities in Arhus, Denmark of 34,910 newborn children shows that 1 per 2130 girls had Turner syndrome [10]. Prevalence in Ukraine the pathology is 1 per 1290 newborn girls [11].

Turner syndrome (TS) results from the absence of some or all of the X chromosome, with a karyotype of 45X in the fully developed syndrome. Because not all genes on the X chromosome are inactivated

normally, the diminished expression of these genes presumably leads to the Turner syndrome phenotype, including short stature, incomplete sexual development and reproductive function, primary ovarian insufficiency and other structural and cognitive abnormalities [1,7,9].

In general, the phenotypic severity of Turner syndrome varies with the extent of X chromosome loss [12]. Affected females can potentially develop a wide variety of symptoms, affecting many different organ systems. Clinical manifestations of Turner syndrome may be categorized as abnormalities affecting multiple organ systems, including the skeletal, cardiovascular and lymphatic, endocrine, gastrointestinal, renal, and the central nervous system. Major morbidities may occur during adult life, and these result in a threefold increase in mortality [6,7,9,13]. Patients with Turner syndrome have an increased incidence of autoimmune disorders (AID), such as Hashimoto's thyroiditis (10-20%), Grave's disease (2-3%), celiac disease, inflammatory bowel disease, and diabetes mellitus [5]. The syndrome is associated with reduced adult height, hyper gonadotropic ovarian failure and infertility or subfertility. Spontaneous pregnancies occur in only 2-7% of women with TS [14,15] and pregnancies in women with 45X monosomy are hardly ever seen. Approximately 50% of affected women are missing an entire X chromosome and have a karyotype of 45X [2,10]. These abnormalities have been hypothesized

to be caused by haploinsufficiency of genes that escape X chromosome inactivation as in the case of normal females [2,7,12]. About 25% have a partial deletion of one X chromosome, while about 20% have varying degrees of mosaicism, most commonly a 45,X/46,XX karyotype [10]. Among TS patients, 45,X/47,XXX karyotype is extremely rare [16]. Women with a Turner mosaic karyotype appear to have a lower risk of obstetrical and cardiovascular complications but should nevertheless undergo the full preconception evaluation [15].

Aim of Our Study

Study was to assess the clinical and the cytogenetic characteristics in patient with Turner syndrome from Lviv region (Ukraine) during 1997-2017.

Materials and Methods

This retrospective study evaluated in 135 female patients aged 1 month–49 years from Lviv region (West Ukraine) who were diagnosed and followed-up at the underwent karyotyping over a period of 20 years (1997–2017) in the laboratory of Medical Genetics Centrum of Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine.

For cytogenetic analysis, peripheral blood was used as biological material. 5 ml peripheral blood was drawn in a sterilized syringe under complete aseptic conditions. Rest of the blood was transferred in heparinized vial. Add 10 ml of either PB-MAX Karyotyping Medium of KARYOMAX or Peripheral Blood Karyotyping Medium to each sterile T-25 flask to be set up for the assay. Add 0.75 ml blood to each tube. Incubate flask in CO₂ incubator for 48-68 hours with caps loose. Add 0.05-0.1 µg/ml COLCEMID to each flask for a 15 minute incubation. After 15 minutes, transfer flask contents to a 15 ml centrifuge tube and spin down at 1200 rpm for 5 minutes. Remove supernatant and re-suspend pellet. Add 10 ml of 0.068 M KCl to pellet and gently mix. Allow to sit at room temperature for 15 minutes Add 0.5 ml of fixative (three parts absolute methanol to none part glacial acetic acid). Gently mix with pipette. Centrifuge for 5 minutes at 1200 rpm. Aspirate off supernatant. Add 10 ml of fixative, mix and let sit at room temperature for 10 minutes. Repeat centrifugation step. Add 5 ml of fixative, mix, and let sit 10 minutes at room temperature. Centrifuge and aspirate off supernatant. Add 5 ml of fixative and

incubate for 10 minutes at 4°C. The cytogenetic karyotype was analyzed on leukocytes cultured from human peripheral blood. A minimum of 30 metaphases was accepted and analyzed through GTG banding with 450- 550 band resolutions observed on microscope (Axiostar plus, Carl Zeiss, Germany)[17,18]. Cytogenetic analyses were performed on phytohaemagglutinin-stimulated lymphocytes according to standard protocol [17,19]. We considered the following data for each patient: age at diagnosis of TS, age at the time of the study, height for review time, medical history and karyotype. Clinical history was recorded and age of spontaneous menarche.

Results

In Lviv region (Ukraine) according to the Statistical Office in January, 2017 population was 2,534,000 persons, including 1,323,300 women (52.2%). Every year in the Lviv region 5-11 girls (rare women) of different ages are found to have Turner syndrome confirmed by karyotyping in the laboratory of Medical Genetics Centrum of Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine. We studied the cohort of patients came from 20 districts of region and Lviv (1997-2017). Our cohort included 135 women with Turner syndrome.

Age of patients ranged from 1 month to 49 years, (median of 16.9 ± 5.3 years).The median age at the time of the study was 20.5 years (18-65). These patients had short stature with a median adult height at 152 cm (126-167) and primary amenorrhea in the majority of cases. Among 63 adolescent patients only 18 (28.6%) had spontaneous menarche.

During this period, patients with suspicion on Turner syndrome turned to the Medical Genetics Centrum of Institute of Hereditary Pathology, National Academy of Medical Sciences of Ukraine, Lviv, Ukraine with various complaints and clinical manifestations. 21 (15.6%) patients were referred to genetics due to low growth, lack of secondary sexual characteristics, with suspicion of pituitary nanism. As a result of cytogenetic research they have been diagnosed with Turner syndrome.

For certitude, the diagnosis can be confirmed using karyotype studies. Karyotype characteristics are reported in Table 1. A monosomy of the X chromosome, was present in 73 (54.1%) of the 135 patient.

Cytogenetic characteristic	Age of diagnosis													
	0-1		1-5		6-11		12-18		19-35		36-49		0-49	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
45,X Monosomy	13	9.6	2	1.5	11	8.1	31	23.0	9	6.7	7	5.2	73	54.1
Only structural rearrangements														
Isochromosome X	-	-	-	-	-	-	1	0.7	5	3.7	3	2.2	9	6.7
Inversion	-	-	-	-	-	-	1	0.7	-	-	-	-	1	0.7
Deletion	-	-	-	-	-	-	1	0.7	-	-	-	-	1	0.7
All structural rearrangements	-	-	-	-	-	-	3	2.2	5	3.7	3	2.2	11	8.1
Mosaicism														
45,X/46,XX mosaicism	3	2.2	1	0.7	6	4.4	3	2.2	7	5.2	1	0.7	21	15.6

Mosaicism Isochromosome X	with	-	-	-	-	1	0.7	11	8.1	-	-	-	-	12	8.9
45,X/47,XXX mosaicism		1	0.7	-	-	1	0.7	1	0.7	2	1.5	1	0.7	6	4.4
Mosaicism with X ring		-	-	-	-	-	-	3	2.2	2	1.5	-	-	5	3.7
Other		-	-	-	-	1	0.7	4	3.0	2	1.5	-	-	7	5.2
All mosaicism forms		4	3.0	1	0.7	9	6.7	22	16.3	13	9.6	2	1.5	51	37.8
All		17	12.6	3	2.2	20	14.8	56	41.5	27	20	12	8.9	135	100

Table 1: Age of diagnosis and characteristic clinical features of Turner syndrome.

Chromosomal analysis was performed to 51 (37.8%) patients diagnosed with Turner syndrome with variable degrees of mosaicism: mosaicism (45,X/46,XX) in 15.6%; mosaicism with polysomy X chromosome (45,X/47,XXX) in 4.4%, 45,X/46,X,i(Xq) in 8.9%, mosaicism 45,X/46,X,r(X) in 3.7% and 7 (5.2%) other cases of mosaicism. Mosaicism monosomy X of 51 patients included 34 (66.7%) with high proportion of 45,X and 17 (33.3%) with low proportion of 45,X.

Among 7 other mosaic cases were cytogenetically characterized 3 cases of three cell lines: mos 45,X [25]/46,XX[3]/46,X,r (X)[2], mos 45,X[22]/46,X,r (X)[5], 46,XX[3], mos 45,X[20]/47,XXX[8]/46,XX[2] and 1 case of marker chromosome mos45,X[24]/46,X,+mar[4]/47,X,+mar1+mar2[2]. We found 2 cases of duplication: mos46,XX,dup(X)(q24qter)[20]/45,X[10] and 45,X[6]/46,XX,dup(X)(p22.3p11.2)[24] and 1 case of deletion- 46,X,del(X)(q26)[18]/46,XX[12].

Only structural X chromosome abnormalities without mosaic forms (Table 1) were found in 11 patients (8.1%) 9 patient with 46,X,i (Xq) (6.7%), one- 46,X,del(X)(q11.1)[30], one- 46,XX,inv(X)(q24q21)[30].

During twenty years (1997-2017) only in 12.6% girls diagnosis of Turner syndrome was established in the 1st year of life, 2.2% in aged 1-5 years, 15.6% in aged 6-11 years old, 40.7% in 12-18 years old, 20% in aged 19-35 years old and 8.9% over 36 years old (Table 1).

The majority of patient with Turner syndrome have been represented at the age category of 12-18 years in 55 people (40.7%). The smallest group of patient appeared to be those at the age 1-5 years in 3 girls (2.2%). Among 12 women over 36 years of age some patients (n=9) were consulted about genetics in connection with endocrine pathology, reproductive anamnesis and other complications. Three patients came to the genetics to confirm the diagnosis that they exhibited over 20 years ago. These women were aged 44-49 years.

Among patients diagnosed of aged 12-18, the ratio of karyotype 45,X was significantly higher (23%) than that of other karyotype groups. The most common karyotype 45,X/46,XX (5.2%) was at aged 19-35, 45,X/46,X,i (Xq) in 8.1% of girls aged 12-18.

Among 135 patients, 113 (84.4%) patients clinically diagnosis was suspected. Small stature, short and broad neck, hypoplasia of the cervical vertebrae, pterygium colli, and gonadal dysgenesis are the most frequent and characteristic signs of the disease. Age of onset is variable according to the presentation: infant: lymphedema of the hands and feet; birth defects, childhood: short stature; adolescence: primary or secondary amenorrhea, gonadal dysgenesis. Clinical features can concern all systems and part of the

bodies: these complex polymalformative syndrome benefits of many symptomatic treatments that have greatly improved patients outcome.

When diagnosis was made from newborn period to the age of 1 year (n=17), the most frequent clinical sign was lymphedema (8 out of 17 patients). In 5 patients, it was isolated, whereas in another 2 it was associated with pterygium colli and in 2 patients it was associated with pterygium colli and coarctation of the aorta. Pterygium colli was found in 7 patients like isolated mark in 2 cases. This was followed by coarctation of the aorta and hypoplasia of chest, which was found in one patient.

At the age of 1-5 years (n=3), the most common symptoms were short neck, pterygium colli, hypertelorism, "Gothic" palate, hypoplasia of the low jaw, epicanthic fold, delayed bone maturation, abnormalities of coagulation factors. At the age of 6-11 years (n=20) short and broad neck, over the curvature of the nails, cubitus valgus, horseshoe kidney, mitral valve prolapse.

During pubertal development (n=56) in adolescence (aged 12-18 years) the most frequent clinical signs were amenorrhea or non-regular menses, delayed puberty, thyroid dysfunction. At the age of 19-35 (n=27) prevailed amenorrhea or non-regular menses, short and broad neck, lack of bodies hair, cubitus valgus, delayed puberty, pectus excavatum, mitral valve prolapse, hypertension, thyroid dysfunction (hypothyroidism). Patients over 36 years of age (n=12) had short and broad neck, thyroid dysfunction, strabismus oculus, excessive distance between the mammary areola, mitral valve prolapse, hypertension, nonregular menses.

Our study group included 40 girls aged 0-11 years (Table 1) and 95 patients aged 12-49. Among 95 patients aged 12-49 mosaic karyotypes was performed in 37 (38.9%), 45,X karyotypes in 47 (49.5%). We observed 11 patients (11.6%) with structural X chromosome abnormalities without mosaic forms in 9 patient with 46,X,i (Xq); one aged 15 years with 46,X,del(X)(q11.1), one aged 14 years with 46,XX,inv(X)(q24q21).

Among 95 patients aged 12-49 pubertal development and spontaneous menarche were timely only in 35 (36.8%) women. Among women aged 12-49 pubertal development and spontaneous menarche were higher in girls with mosaic karyotypes in 20 (54.1%) out of 37, but up to 25.5% of girls with 45,X karyotypes (12 of 47) have some signs of spontaneous puberty. Spontaneous pubertal and spontaneous menarche occurred in 3 out of 11 (27.3%) subjects with structural X chromosome abnormalities without mosaic forms. Spontaneous menarche was more common in patients with mosaicism than in those with 45,X karyotypes, and concluded that the additional X

chromosome likely has a significant influence on the progression of puberty.

Girls with Turner syndrome had problems with menarche. During follow up among 95 patients, 37 patients were found to have regular menses from 5-10 years after the onset of menarche, 17 patients developed secondary amenorrhea 1-4 years after the onset of menarche, 28 patients developed oligomenorrhea, 11 patients had non-regular menses and 2 had primary continued amenorrhea.

We studied clinical analysis of all systems of our patients according cytogenetic characteristic (Table 2). Women with Turner syndrome are more likely to have abnormality of skeletal system in 78 (57.8%), including short neck and hypoplasia of the low jaw, prevalence in patients with 45,X karyotypes (35.6%) and 45,X/46XX mos (8.9%),

mos with X ring (3.7%). Thyroid dysfunction is common in our women with Turner syndrome, with a prevalence approaching 57% (77 cases) in patients with 45,X/46,XX mos (10.4%), 45,X/47,XXX mos (3.7%), mosaicism with Isochromosome X (6.7%). In our study only one women aged 44 with 45X karyotypes had diabetes mellitus which developed at the age of 36 years after nervous stress. Lymphedema of the hands and feet was prevalence in newborns with 45,X karyotypes (7.4%). Urinary system, including renal cysts both or one kidney, horseshoe kidney, unilateral renal agenesis; pelvis and ureters alterations, were performed in patients with 45,X karyotypes (20%). Rare pathology of this system met in women with 45,X/46,XX mos (0.7%), 45,X/47,XXX mos (0.7%). Pathology of another organs and systems (cardiovascular, respiratory, and gastrointestinal) were between 12.6%-16.3% (Table 2).

Cytogenetic characteristic	Pathology of organs and systems																	
	Lymphedema		Endocrine		Skeletal system		Urinary		Cardio-vascular		Respi-ratory		Gastro-intestinal		Nervous		Repro-ductive	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
45,X Monosomy	10	7.4	39	28.9	48	35.6	27	20	14	10.4	13	9.6	12	8.9	18	13.3	42	31.1
45,X/46,XX mos	3	2.2	14	10.4	12	8.9	1	0.7	1	0.7	1	0.7	1	0.7	2	1.5	10	7.4
45,X/47,XXX mos	1	0.7	5	3.7	3	2.2	2	1.5	-	-	-	-	1	0.7	1	0.7	3	2.2
Mos with X ring	-	-	3	2.2	5	3.7	-	-	-	-	-	-	-	-	3	2.2	3	2.2
Isochromosome X	-	-	6	4.4	2	1.5	1	0.7	3	2.2	3	2.2	-	-	1	0.7	9	6.7
Mos with Isochromosome X	-	-	9	6.7	4	3.0	-	-	4	3.0	1	0.7	-	-	3	2.2	1	0.7
Other	-	-	1	0.7	4	3.0	-	-	-	-	1	0.7	3	2.2	3	2.2	1	0.7
All	14	10.4	77	57	78	57.8	31	23	22	16.3	18	13.3	17	12.6	57	23	69	51.1
Mos- Mosaicism																		

Table 2: Cytogenetic characteristic and clinical features of Turner syndrome.

Structure of clinical features in patients with Turner syndrome had frequently demonstrated: bronchitis, nasopharyngitis, alveolitis, pneumonia and cholelithiasis, gastritis, peptic ulcers of stomach and duodenum, gastro-esophageal reflux, abdomen pain, pancreatitis. Cardiovascular comorbidities at the time of evaluation were mitral valve prolapse, hypertension, aortic valve disease, bicuspid aortic valve, and aortic coarctation, anomalous of the pulmonary veins.

Nervous system was rare affected in women with 45,X/47,XXX mos (0.7%) and isochromosome X (0.7%). Reproductive system was disturbed more likely in women with 45,X karyotypes (31.1%), 45,X/46,XX mos (7.4%), Isochromosome X (6.7%).

Conclusion

1. We evaluated 135 female patients with Turner syndrome aged 1 month–49 years from Lviv region (West Ukraine) who were diagnosed and followed-up at the underwent karyotyping over a period of 20 years. The average age at diagnosis was 16.9 ± 5.3 years.

2. Cytogenetic Characteristics of the Turner syndrome patients showed that most of girls with TS (54.1%) had karyotype 45,X mosaicism monosomy X was found in 51 patients (37.8%). Only

structural X chromosome abnormalities without mosaic forms were found in 11 patients (8.1%).

3. In 12.6% girls diagnosis was established in the 1st year of life and the most frequent clinical sign was lymphedema was prevalence in newborns with 45,X karyotypes (7.4%).

4. Pubertal development and spontaneous menarche were higher in girls with mosaic karyotypes in 54.1%, but up to 25.5% of girls with 45,X karyotypes have some signs of spontaneous puberty.

5. Women with Turner syndrome are more likely to have abnormality of skeletal system in 78 (57.8%) and pathology of endocrine system 57% (77 cases), especially thyroid dysfunction and reproductive system in 51.1% (69 cases).

In Lviv region (West Ukraine) the highest incidence of primary diagnosis of Turner syndrome is after 12 years old. The largest proportion of patients with TS has a karyotype with monosomy X.

During last 5-10 years diagnosis of Turner syndrome is more frequently in aged 1 year. The high median aged of diagnosis in Lviv region (West Ukraine) during 1997-2017 twice more than in Europe.

This can be explained that first clinical features appeared during puberty.

References

1. McCarthy K, Bondy CA (2008) Turner syndrome in childhood and adolescence. *Expert Rev Endocrinol Metab* 3: 771-775.
2. Noordman I, Duijnhouwer A, Kapusta L, Kempers M, Roeleveld N, et al. (2018) Phenotype in girls and women with Turner syndrome: association between dysmorphic features, karyotype and cardio-aortic malformations. *Eur J Med Genet*.
3. Van den Hoven AT, Duijnhouwer AL, Eicken A, Aboulhosn J, de Bruin C, et al. (2017) Adverse outcome of coarctation stenting in patients with Turner syndrome. *Catheter Cardiovasc Interv* 89: 280-287.
4. Henry H (1972) Classic pages in obstetrics and gynecology : A syndrome of infantilism, congenital webbed neck, and cubitus valgus. *Endocrinology* 23: 566-574.
5. Aversa T, Gallizzi R, Salzano G, Zirilli G, De Luca F, et al. (2018) Atypical phenotypic aspects of autoimmune thyroid disorders in young patients with Turner syndrome. *Ital J Pediatr* 44: 12.
6. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, et al. (2017) Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *International Turner Syndrome Consensus Group. Eur J Endocrinol* 177: G1-G70.
7. Stochholm K, Juul S, Juul K, Naeraa RW, Gravholt CH (2006) Prevalence, incidence, diagnostic delay and mortality in Turner syndrome. *J Clin Endocrinol Metab* 91: 3897-3902.
8. Gravholt C (2004) Epidemiological, endocrine and metabolic features in Turner syndrome. *Eur J Endocrinol*. 151: 657-687.
9. Lleo A, Moroni L, Caliri L, Invernizzi P (2012) Autoimmunity and Turner's syndrome. *Autoimmun Rev* 11: A538-A543.
10. Nielsen J, Wohler M (1990) Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus Denmark. *Birth Defects Orig Artic Ser* 26: 209-223.
11. Zelinska N, Shevchenko I, Globa E, Pogadaeva N (2015) Nationwide study of Turner syndrome in Ukraine [Electronic resource]. *ESPE Abstracts, 36th Annual Meeting of the European Society for Pediatric Endocrinology, Barcelona, Spain*.
12. Zheng J, Liu Z, Xia P, Lai Y, Wei Y, et al. (2017) Clinical manifestation and cytogenetic analysis of 607 patients with Turner syndrome. 34: 61-64.
13. Bucerzan S, Miclea D, Popp R, Alkhzouz C, Lazea C, et al. (2017) Clinical and genetic characteristics in a group of 45 patients with Turner syndrome (monocentric study). *Ther Clin Risk Manag* 13: 613-622.
14. Folsom LJ, Uqua JS (2015) Reproductive Issues in Women with Turner Syndrome. *Metab Clin North Am* 44: 723-737.
15. Bouet PE, Godbout A, Hachem H, Lefebvre M, Bérubé L, et al. (2016) Fertility and Pregnancy in Turner Syndrome. *J Obstet Gynaecol Can* 38: 712-718.
16. Martin RJ, Smith G, Hughes J, Morrison PJ (2018) Incidence, puberty, and fertility in 45,X/47,XXX mosaicism: Report of a patient and a literature review. *Am J Med Genet*.
17. Shaffer LG, Jordan MG, Schmid M (2013) An international system for human cytogenetic nomenclature. *Karger* p. 137.
18. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp Cell Res* 20: 613-616.
19. Seabright M (1971) Rapid banding technique for human chromosomes. *Lancet* 2: 971-972.