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Original scientific article

CHROMOSOMAL ANALYSIS IN 110 PATIENTS WITH PRIMARY AMENORRHEA

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Objects: This study was undertaken to investigate frequency and the type of chromosomal aberrations which are causing primary amenorrhea in our patients. Methods: The study subjects included 110 patients referred with primary amenorrhea for cytogenetics investigation and counseling. Primary amenorrhea was defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older, or aged 16 years or older if secondary sexual characteristics were present. Peripheral blood samples were processed using standard techniques. All spacimens were G-banded using tripsin-Giemsa. For each case, 22 metaphase spreads were analyzed and when mosaicism was suspect 100 metaphases were examined. Results: We have examined cytogenetically 110 patients with primary amenorrhea. Chromosomal aberrations were detected in 21 cases (19,1%). Male karyotype (46,XY) was found in seven cases, as well as monosomy X (45,X). In three cases isochromosome X (46,XiXq) was detected. We found two cases of mosaic karyotype 46,XX/45,X and X chromosome trisomy (47,XXX) and mosaic karyotype 45,X/46,XiXq in one case each. Conclusion: Chromosome aberrations are one of the main causes of primary amenorrhea. The search for genetic component is the utmost importance for diagnosis, risk assessment and genetic counseling.

Key words: primary amenorrhea, chromosome aberrations, X chromosome aneuploidies

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INTRODUCTION

The World Health Organization has estimated 15% of the human population as being infertile and amenorrhea as the sixth largest major cause of female infertility. Among the general population amenorrhea seemed to have affected 2-5% of all woman of childbearing age (SUSHEELA, 2019).

Primary amenorrhea is the complete absence of menstruation. It is defined as a failure of menarche, associated with undeveloped secondary sexual characteristics by age 14, or failure of menarche with well developed secondary sexual characteristics by age 16 (ALI *et al*., 2018).

The causes of primary amenorrhea can be categorized as the functional or anatomic defect of the hypothalamus, the functional or anatomic defect of the pituitary, the functional or anatomic defect of the uterus or ovaries, and genetic defect which may be either at chromosomal or at a gene level (PAL *et al*., 2019).

Previous reports suggest that chromosomal aberrations are the second most common cause of amenorrhea (GHOSH *et al*., 2018). The percentage of chromosomal abnormalities reported varies greatly, from 6,6% to 54.56% for primary amenorrhea (JOSEPH & THOMAS, 1982, TEN *et al*.,1990, BUTNARIU *et al.,* 2011).

This study was undertaken to investigate frequency and the type of chromosomal aberrations which are causing primary amenorrhea in our patients.

MATERIALS AND METHODS

Patients

The study subjects included 110 patients referred with primary amenorrhea for cytogenetics investigation and counseling to the Department for Cytogenetics, Clinic for gynecology and obstetrics, Clinical Center of Serbia, Belgrade, from 2002 to 2012.

Patients were referred from Department for gynecological endocrinology, at the same clinic, after exclusion of non-genetic causes. The diagnosis of primary amenorrhea was ascertained at the patients initial visit. Primary amenorrhea was defined as the absence of menstruation and secondary sexual characteristics in phenotypic women aged 14 years or older, or aged 16 years or older if secondary sexual characteristics were present. Physical examination was performed to identify any secondary sexual characteristic or syndromic features. Laboratory investigation and clinical information were obtained from clinical records. The age group of the subjects ranged from 14 to 32 years.

Methods

About 5 ml of peripheral blood was collected from each patient in a heparinized syringe. Cultures of peripheral blood lymphocytes in RPMI 1640 basal medium and 10% fetal calf serum were treated with 0,1 μg/ml of colcemid after 72h incubation period and then metaphase chromosomes were spread and stained using standard G-banding technique (MOORHEAD *et al*., 1960, SEABRIGHT, 1971). For each case, 22 metaphase spreads were analyzed and when mosaicism was suspect 100 metaphases were examined. The International System for Human Cytogenetic Nomenclature was used to describe karyotypes (ISCN, 1995, ISCN, 2005).

RESULTS

For the period of ten years we have examined 110 patients with primary amenorrhea. Age at the referral to our clinic ranged from 14 to 32 years.

A pathological or male karyotype was detected in 21 (19,1%) cases (Table 1.).

Table 1. Cytogenetic findings in primary amenorrhea

Karyotype	Number of cases (%)
46,XX	89 (80,9%)
46,XY	7 (6,4%)
45,X	7 (6,4%)
46,X i(Xq)	3 (2,7%)
46,XX/45,X	2 (1,8%)
47,XXX	1 (0,9%)
45,X/ 46,X i(Xq)	1 (0,9%)
Total	110 (100%)

Male karyotype 46,XY was present in seven cases (6,4%): five had pure gonadal dysgenesis, one was suspected to have pseudohermaphroditism, and at one patient physical and ultrasound examination revealed absence of uterus and gonads, and a small tubular soft tissue posterior to the urinary bladder suggestive of underdeveloped Mullerian duct..

The most frequent aberrant karyotype were X chromosome aneuploidies, in 10 cases (9,1%), including monosomy X (Turner syndrome, n=7), 46,XX/45,X mosaicism (n=2) and 47,XXX (triple X syndrome, n=1). The percentage of mosaicism was 8% and 23%. Two patients with mosaicism were considered to have normal secondary sexual characteristics, while other patients with X chromosome abnormalities showed poor development of breasts, genital organs or secondary sexual characteristics. Two of them, with monosomy X, had clinical characteristics of Turner syndrome.

Structural anomalies of the X chromosome were detected in three cases (2,7%), and all had isochromosome of long arm of X chromosome, 46,X i(Xq).

We also found case of mosaicism 45,X/ 46,X i(Xq).

DISCUSSION

A large number of survey have been undertaken worldwide to ascertain the frequency and nature of chromosomal aberrations in primary amenorrhea.

In Indonesia, ALI *et al*. (2018) found a prevalence of chromosomal aberrations of 22.8% in 79 women, and KOPPAKA *et al*. (2019) in India reported a prevalence of 31.2% in 3776 patients, while STOYANOVA *et al*. (2015) revealed a prevalence of 32,6% in Bulgarian population in 141 woman with primary amenorrhea.

Some studies have reported higher prevalence, like CORTÉS-GUTIÉRREZ *et al.* (2007) that reported a prevalence of chromosomal aberrations of 41.7% in 187 women with primary amenorrhea in Mexican population, and BUTNARIU *et al.* (2011) that found a prevalence of 54,6% in Romanian population in 493 patients.

In contrary, some other studies have reported lower prevalence of chromosomal aberrations: in Saudi Arabia, AL-JAROUDI (2019) found a prevalence of 19% in 42 patients; in Turkish population, KARA *et al.* (2012) revealed a prevalence of 14.3% in 89 patients; and in India, PAL *et al*. (2019) reported a prevalence of 13.2% in 174 women with primary amenorrhea referred to genetic laboratories by clinicians.

Prevalence following this study of 19,1%, was in accordance to the world wide estimated range for chromosomal abnormalities among primary amenorrhea patients.

Chromosomal aberrations in primary amenorrhea could be grouped as the X numerical, X structural and 46,XY female, the sex reversal or pseuohermaphroditism condition. Their frequencies from the literature are X numerical (20%-50%), X mosaicism (21%-43%), X structural (22%-24%) and 46,XY female (4%-34%) (EL-DAHTORY, 2012, DEMIRHAN *et al*., 2014, STOYANOVA *et al.* 2015).

In our study monosomy X and male karyotype were the most common findings.

Studies have demonstrated that a female phenotype can occur in XY embryo when gene encoding for testis determining factor (TDF) or other genes in the testes determining pathway are lost, mutated or compromised. Consequently, these cases need to be further assessed for any mutation of SRY and SF1 gene and also in the other genes which are responsible for male karyotype in phenotypic female with primary amenorrhea (KORGAONKAR *et al*., 2019).

In 10%–15% of cases of female sex reversal, mutation of the SRY gene was seen. The remaining cases may be due to mutation of other genes involved in sex differentiation pathways such as WT1, DHH, NR5A1, SOX9, GATA4, FOG2/ZFPM2, and MAP3K1, as well as chromosomal adjustment including deletions of chromosome 9p and duplications of Xp22 (PAL *et al*., 2019, SUSANTI *et al*., 2020).

It is important to determine the karyotype, as the presence of Y chromosome is an indication for the surgical removal of gonads, because there is 30% risk of malignant gonadal line alterations in patients with 46, XY complete gonadal dysgenesis (ANAGANI *et al*., 2017, SUSANTI *et al*., 2020).

Other common finding in our study was monosomy X (Turner syndrome), the karyotype usually seen in primary amenorrhea. These women may have Turner stigmata: short stature, webbing of neck, hypoplasia or aplasia, nipples far apart, barrel shaped chests, cubitus valgus, pigmented nevi, streak gonads or hypoplastic uterus. The short stature observed in majority of the cases of Turner syndrome appears to be triggered by the lack of a second sex chromosome. In Turner syndrome cases, the genes involved in gonadal function are located on the proximal part of Xp and also on the distal part of the Xq, where as the genes whose absence is responsible for somatic features of the syndrome may be distributed along the length of Xp and the middle section of Xq (q21-q26).17 (DUTTA *et al*., 2013).

Studies reported that haploinsufficiency of the short-stature homeo box (SHOX) gene mapped on the pseudoautosomal region of the X and Y chromosomes has been causally responsible (ABIR *et al*., 2001).

Patients with pure monosomy of X chromosome are invariably infertile, but pregnancy is possible in patients with X-chromosome abnormalities, especially when there is a low percentage of mosaicism (WONG & LAM 2005).

The structurally abnormal X chromosome may be inactivated and minimize the disturbance of cellular function. Moreover, the phenotype may be indirectly influenced as per the size, and the loss/gain/altered genetic function, in the deleted or duplicated segments in X (CHARLES & NEIL 1982, RAJANGAM & NANJAPPA 2007). In our study we have found three cases of isochromosome Xq, but no translocations or other structural anomalies.

Structural abnormality leading to primary amenorrhea mostly is isochromosome of the long arm of X, iXq. This leads to the partial monosomy of the genes present on Xp and partial trisomy of genes present on Xq. Females with 46,X,i(Xq) karyotype have been found to manifest streak gonads. Complete ovarian failure and partial ovarian failure have also been reported in 91% and 9% of cases with i(Xq) individuals, respectively. Besides, short stature and Turner syndrome stigmata are found to be frequent with almost complete lack of gonadal development in 46,X,i(Xq) females (MALLA *et al*., 2016).

We have also found one case of triple X. It has been suggested that girls with karyotype 47,XXX have a higher incidence of ovarian failure (DEWHURST, 1978).

It has also been reported that premature ovarian failure may be secondary to X-chromosome deletions or translocations. Reports of patients with premature ovarian failure and Xq deletions suggest that there is a gene (POF1) localized to Xq21.3-q27 or within Xq26.1-q27 and a gene (POF2) localized to Xq13.3-q21.1, as well as a gene on a short arm in a region Xp11 (DUTTA *et al*., 2013).

Previous studies also showed that serum follicle stimulating hormones and luteinizing hormones are always increased in primary amenorrhea patients with chromosomal abnormalities (EL-DAHTORY, 2012).

Karyotyping aids in confirmation of diagnosis, a better phenotype-genotype correlation to understand clinical heterogeneity, and in genetic counseling. Genetic counseling should include the risk of gonadal malignancy for patients with XY gonadal dysgenesis, the risk of premature menopause for patients with TS and the use of hormonal replacement therapy, and the possibility of infertility in the future children of patients with mosaic TS cases (DUTTA *et al.*, 2013).

CONCLUSIONS

Chromosome aberrations are one of the main causes of primary amenorrhea. The search for genetic component is the utmost importance for diagnosis, risk assessment and genetic counseling.

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REFERENCES

ABIR R., B. FISCH, R. NAHUM, R. ORVIETO, S. NITKE, Z. BEN RAFAEL (2001): Turner's syndrome and fertility: current status and possible putative prospects. Human Reproduction, *7*: 603-610.

ALI A., R. INDRIYATI, T. WINARNI, S. FARADZ (2018): Cytogenetic Analysis and Clinical Phenotype of Primary Amenorrhea in Indonesian Patients. Journal of Biomedicine and Translational Research, *4*(1): 22-27.

AL-JAROUDI D., A. HIJAZI, M. BASHIR, H. HEENA, S.A. TASHKANDI (2019): Chromosomal aberrations in women with primary and secondary amenorrhea: A cross-sectional study. J Obstet Gynaecol Res, *45*(8): 1497-1505.

ANAGANI M., P. AGRAWAL, B. RADHIKA, D. SRIDEVI, M KAREE (2017) Primary Amenorrhea - A One Year Review. Obstet Gynecol Int J, *6*(1): 00189.

BUTNARIU L, M. COVIC, I. IVANOV, C. BUJORAN, M. GRAMESCU, E. VLAD GORDUZA (2011): Clinical and cytogenetic correlation in primary and secondary amenorrhea: retrospective study on 531 patients. Revista Română de Medicină de Laborator, *19*: 149–160.

CHARLES R.K., S.R. NEIL (1982): Pregnancy in a patient with 47,XX, i(Xq) karyotype. J Med Genet, *19*: 467-468.

CORTES-GUTIERREZ E.I., M.I. DAVILA-RODRIGUEZ, J. VARGAS-VILLARREAL, R.M. CERDA-FLORES (2007): Prevalence of chromosomal aberrations in Mexican women with primary amenorrhoea. Reprod Biomed Online, *15*(4): 463-467.

DEMIRHAN O., N. TANRIVERDI, E.TUNC, N. INANDIKLIOGLU, D. SULEYMANOVA (2014): Frequency and types of chromosomal abnormalities in Turkish women with amenorrhea. J Pediatr Adolesc Gynecol, *27*: 274–277.

DEWHURST J. (1978): Fertility in 47,XXX and 45,X patients. J Med Genet, *15*: 132-135.

DUTTA U.R., R. PONNALA, V.K. PIDUGU, A.B. DALAL (2013): Chromosomal abnormalities in amenorrhea: A retrospective study and review of 637 patients in south India. Arch Iran Med, *16*(5): 267 – 270.

EL-DAHTORY F. (2012): Chromosomal abnormalities and hormonal disorders of primary amenorrhea patients in Egypt. Indian J Hum Genet, *18*(2): 183–186.

GHOSH S., S. ROY, P. PAL, A. DUTTA, A. HALDER (2018): Cytogenetic analysis of patients with primary amenorrhea in Eastern India. J Obstet Gynaecol, *38*: 270–275.

ISCN (1995): An international system for human cytogenetic nomenclature (1995): recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, Mitelman F. (ed). Karger, USA.

ISCN (2005): International System of Human Cytogenetic Nomenclature, Shaffer LG, Tommerup N (eds). S Karger AG, Basel.

JOSEPH A., I.M. THOMAS (1982): Cytogenetic investigations in 150 cases with complaints of sterility or primary amenorrhea. Hum Genet, *61*: 105–109.

KARA N., S. TURAL, M. ELBISTAN, N. KARAKUS, D. GUVEN, I. KOCAK (2012): Cytogenetic Findings of Patients with Amenorrhea in Turkish Population: A Retrospective Study. Int J Hum Genet, *12*(2): 87-92.

KOPPAKA N.T., S.K. VIRULKAR, D.S. CHAVAN, R.C. DALVI, N. GUPTA, S. MANDAVA (2019): Specific Chromosomal Aberrations in Primary Amenorrhoea: Study on 3776 Cases from Indian Population. J Obstet Gynecol India, *69*:457–461.

KORGAONKAR S., S. DHANGAR, V. KULKARNI, L. KERKETTA, B.R. VUNDINTI (2019): Chromosomal aberrations in primary amenorrhea: A retrospective study. J Hum Reprod Sci, *12*: 92-7.

MALLA T.M., F.A. DAR, A.A. PANDITH, M.H. ZARGAR (2016):Frequency and pattern of cytogenetic alterations in primary amenorrhea cases of Kashmir, North India. The Egyptian Journal of Medical Human Genetics, *7*: 25–31

MOORHEAD P.S., P.C. NOWELL, W.J. MELLMAN, D.M. BATTIPS, D.A. HUNGERFORD (1960): Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res, *20*: 613-616.

PAL A.K., P.S. AMBULKAR, B.R. SONTAKKE, S.S. TOLHAR, P. BOKARYA, V.K. GUJAR (2019): A study on chromosomal analysis of patients with primary amenorrhea. J Hum Reprod Sci, *12*: 29-34.

RAJANGAM S., L. NANJAPPA (2007): Cytogenetic studies in amenorrhea. Saudi Med J, *28*(2): 187-192.

SEABRIGHT M. (1971): A rapid banding technique for human chromosomes. Lancet, *2*: 971-972.

STOYANOVA V., A. LINEV, H. IVANOV, T. VACHEV (2015): Cytogenetic analysis of patients with primary amenorrhea. Akusherstvo i ginekologiia, *54*(3): 13-17.

SUSANTI N.M.I.D., I. MULYANTOROL, D. PUSPASARI, N.A. LISTYASARI, S.M.H. FARADZ (2020): Unraveling of Diagnostic Odyssey in A Girl with Primary Amenorrhea: A case report. Journal of Biomedicine and Translational Research, *6*(1): 18-22.

SUSHEELA B.R. (2019): Etiological factors of primary amenorrhoea: A prospective study. International Journal of Clinical Obstetrics and Gynaecology, *3*(4): 228-230.

TEN S.K., Y.M. CHIN, P.J. NOOR, K. HASSAN (1990): Cytogenetic studies in women with primary amenorrhea. Singapore Med J, *31*: 355-359.

WONG M.S.F., S.T.S. LAM (2005): Cytogenetic analysis of patients with primary and secondary amenorrhea in Hong Kong: retrospective study. Hong Kong Med J, *11*(4): 267-272.

ANALIZA HROMOZOMA 110 PACIJENTKINJA SA PRIMARNOM AMENOREJOM

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Izvod

Istraživanje smo sproveli u cilju utvrđivanja učestalosti i tipa hromozomskih aberacija koje uzrokuju primarnu amenoreju kod naših pacijentkinja. Studijom je obuhvaćeno 110 pacijentkinja sa primarnom amenorejom, kojima je rađena analiza kariotipa u cilju dijagnostike i genetičkog savetovanja. Primarna amenoreja definisana je kao odsustvo menstruacije i sekundarnih seksualnih karakteristika kod žena starih 14 i više godina, ili 16 i više godina ukoliko su prisutne sekundarne seksualne karakteristike. Uzorci periferne krvi obrađeni su standardnim tehnikama. Nakom bojenja preparata G-tehnikom traka, u svakom uzorku je analizirano po 22 ćelije u metafazi. U slučaju sumnje na mozaicizam analizarano je po 100 ćelija u metafazi. Analizirali smo kariotip 110 pacijentkinja sa primarnom amenorejom. Hromozomske aberacije su detektovane u 21 slučaju (19,1%). Muški kariotip i monozomija X hromozoma nađeni su u po sedam slučajeva. U tri slučaja detektovan je izohromozom X (46,XiXq). U dva slučaja smo našli mozaični kariotip 46,XX/45,X, a u po jednom trizomiju X hromozoma (47,XXX) i mozaični kariotip 45,X/46,XiXq. Hromozomske aberacije su jedan od najčešćih uzroka primarne amenoreje. Traganje za genetičkom komponentom je od velike važnosti za dijagnozu, procenu rizika i genetičko savetovanje.

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