CYTOGENETIC ABNORMALITIES IN PATIENTS WITH PRIMARY AMENORRHEA

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ABSTRACT

Objective: To determine pattern of different cytogenetic abnormalities found in patients of primary amenorrhea.
Study Design: Cross sectional study.
Place and Duration of Study: Chromosomal analysis was carried out on patients who were sent for diagnosis and evaluation of primary amenorrhea to Armed Forces Institute of Pathology, from Mar 2013 to Mar 2017.
Methodology: Chromosomal culture and karyotype analysis was done on peripheral blood of 260 patients sent for cytogenetic testing. Sample was collected in sodium Heparin and after 72 hours, 20 metaphases were observed.
Results: A total of 260 patients with complaints of primary amenorrhea (PA) were karyotyped. Patients who had karyotype abnormalities were 48 (18.5%). Most common abnormality was Turners syndrome present in 30 individuals (11.5%) including mosaic pattern which was present in 8 patients. Male karyotype was present in 8 (3.07%) patients. Structural chromosomal abnormality Isochromosome (iXq) was seen in 4 patients. One patient had 46X with del Xp (p 1.1). One patient had 46XX t (4; 14). Marker chromosome was seen in one patient. Two patients had 47 XXX.
Conclusion: Cytogenetic disorders have a considerable impact on individuals, their families and on society. Early diagnosis of such disorder is imperative. All the cases of primary amenorrhea should have cytogenetic testing.

Keywords: Cytogenetics, Karyotyping, Pakistan, Primary amenorrhea.

INTRODUCTION

Menstruation is a sign of female puberty, which is an important milestone in a woman's life. In United States, average menarche age is 12.6 years with a range of 9-15 years. Menarche age varies in different ethnic groups, however, with every passing decade; females are experiencing it in early age. In case, if a female does not menstruate by age of 16 years, it shows abnormality and must be investigated[1]. Incidence of primary amenorrhea (PA) in USA is less than 1%.

Menarche and regular menstrual cycles require normal working of major body systems like, endocrine axis, thyroid, pancreatic function etc. Hypo or hyper functioning of these systems may cause amenorrhea. Even a severe systemic illness or anorexia nervosa may lead to this condition. Pituitary/hypothalamic axis disorder (27.8%) is also one of the common causes of amenorrhea which decreases gonadotrophin pulsation and results in absent LH surge. These may be caused by eating disorders, strenuous exercise, physical and mental stresses or psychiatric disorders[2].

Two other important reasons of primary amenorrhea are improper functioning of gonads (50.4%); and outflow tract abnormalities (21.8%), which make almost fifty percent of PA cases. This category of etiology often comes from abnormal sex chromosomes. Amongst the chromosomal abnormalities, Turners syndrome is most common, comprising up to 29.7% of all primary amenorrhea cases followed by less common cases with Isochromosome X, XY and other rare karyotype[3].

Turner syndrome is named after Henry Turner from Illinois, who described it in 1938. It is caused by the absence of one complete or partial copy of the X chromosome in few or all of the cells. The abnormal cells may contain only one X chromosome (monosomy) (45,X) or may have one of other types of partial monosomy, like

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a deletion of the short p arm of one X chromosome (46,X.del (Xp)) or the presence of an Isochromosome with two q arms (46,X,i(Xq)). In mosaic individuals, cells with X monosomy (45, X) may occur along with cells that are normal (46 XX) or cells that have partial monosomy[4].

In most of the cases, where only one chromosome is present, it is inherited from mother. This may be due to a nondisjunction in the father[4,5]. Meiotic errors that produce X chromosome with p arm deletions or abnormal Y chromosomes are also mostly found in the father. Isochromosome or ring chromosome X on the other hand are formed equally often by both parents. Overall, the functional X chromosome usually comes from the mother[5].

Turner syndrome is usually a sporadic event. For the parents of an individual with Turner syndrome, the risk of recurrence is not increased for subsequent pregnancies. Rare exceptions may include the presence of a balanced translocation of the X chromosome in a parent, or where the mother has 45 X mosaicism restricted to her germ cells.

As a chromosomal condition, there is no cure for Turner syndrome. However, symptomatic treatment may be given such as Growth hormone to attain good adult height, Estrogen replacement therapy for development of secondary sexual characteristics. Estrogens are crucial for maintaining good bone integrity, cardiovascular health and tissue. Assisted reproductive techniques may be used in selective cases[6].

Females with Y chromosomes are another group which may present with PA. Most of these patients have female external genitalia as Mullerian inhibitory factor and testosterone are not being produced. Many of these patients may have ambiguous genitalia and are diagnosed early in life. Still a number of patients do present in adult life with primary amenorrhea. Most females with Y chromosomes have atrophied gonads in their pelvis and they are prone to develop tumors that should be surgically removed[7].

Severe unintended psychological harm may be caused to such females, when they are informed about presence of male karyotype after spending many years as female. Psychiatric and social impact of such disorders is very high, yet fewer patients are seeking medical help. Consultation and counseling is of utmost importance in all such cases. Counseling should involve not only the persons suffering from such disorders but should also include parents, siblings and close family members.

Karyotyping can determine the numerical and structural abnormalities in such disorders. Thus, it is one of the standard diagnostic procedures for identifying chromosomal abnormalities involved in this disorder[8,9,10].

METHODOLOGY

A cross sectional study was carried out on 260 patients who were referred to Armed Forces Institute of Pathology, Rawalpindi (AFIP) over a period of five years i.e. from March 2013 to March 2017 with complaints of primary amenorrhea. Sample size was calculated by using WHO sample size calculator at 95% confidence interval, 5% alpha error and prevalence of chromosomal abnormalities are 20%[11]. Patients were from different ethnic origin mostly from northern part of Pakistan. Detailed history and examination of patients was done. Non probability consecutive sampling was done after taking informed consent. All female patients between the ages of 10-45 were included.

Sample was collected in sodium heparin after proper history taking and informed consent. About 1 ml of blood was supplemented with Roswell Park Memorial Institute (RPMI) medium, fetal bovine serum of PHA (Phytohemaglutinin), fungizone and penicillin were also added and incubated at 37°C with 5% CO_2. After 72 hour of incubation, ethidium bromide (1mg/ml) was added followed by the Colchicine (1mg/ml) to arrest cycle at metaphase stage and again incubated for another one hour. We obtained cells by applying a hypotonic solution of 0.075M KCl at 37°C for 20 minutes. This was followed by

fixation and rinsing three times, using Carnoy's fixative (methanol and acetic acid in a ratio of 3:1). Finally slides were made and stained with Leishman dye[12].

Twenty metaphase spreads were investigated for each case. Slides were examined and analyzed. Karyotype reports were based on the International System for Human Cytogenetic Nomenclature recommendations (ISCN, 2013)[13].

MS Office Excel (2007) was used for data analysis. Mean and SD (Standard deviation) was calculated for quantitative variable like age. Frequencies and percentages were calculated for qualitative variables like chromosomal abnormalities.

RESULTS

Out of 260 patients, age ranging, from 10-35 years of age. Average age of patients at the time of presentation was 15.5 ± 4.24 years.

Results of karyotype are shown in table-I. In our study, normal karyotype was found in 212 cases (81.5%) of PA. suggesting non chromosomal causes of amenorrhea, such as hypothalamic disorders, hypothyroidism, anorexia nervosa or other systemic diseases.

Abnormal karyotype was found in 45 (18.5%) cases. Twenty five (25) patients had numerical chromosomal abnormalities which included 22 patients with monosomy XO, 2 patients with 47XXX and one patient with marker chromosome.

Structural chromosomal abnormality was present in 23 patients. These cases included mosaic XO/XX in 8 cases, presence of XY chromosomes in 8 cases, Isochromosome in 4 cases, one patient had 46X with del Xp (p 1.1), one patient had 46XX t (4; 14) and one patient had 46X with del Y q arm.

In our study, most common disorder found was Turners syndrome 45X0 followed by mosaic Turner. One patient had XY chromosome with deletion of q arm.

We compared our results with other studies as tabulated in table-II. It showed similarities and interesting differences amongst various studies. Structural and numerical chromosomal abnormalities of other studies are noted and compared. Only one study has mentioned about marker chromosome and one other study about Isochro-

Table-I: Frequency of different cytogenetic abnormalities in primary amenorrhea individuals.

Chromosomal abnormalities	Karyotype	No. of Cases (n=260)	Frequency
Abnormal karyotype		48	18.5
Monosomy X	XO	22	8.5
Presence of XY chromosomes	XY	8	3
Isochromosome	46XiXq	4	1.5
Mosaic Turner	XO/XX	8	3
46X with del Xp(p1.1)		1	0.38
46XX t(4;16)		1	0.38
46X with del Y q arm		1	0.38
47XXX	47XXX	2	0.77
Marker chromosome		1	0.38

Table-II: Comparison of cytogenetic patterns with different studies.

Results	Present study	Safaei *et al*[11]	Farnaz *et al*[16]	Kong *et al*[17]	Butnariu *et al*[18]	Tahir *et al*[19]
Frequency of abnormal karyotype	48 (18.5%)	44 (20%)	44 (24.44)	10 (58.8%)	269 (54.56)	38 (35.19%)
Turner and mosaic Turner	30 (11.5%)	23 (52.27%)	28 (63.6%)	29 (50%)	221 (82.15)	31 (28.69%)
46XY	8 (3%)	12 (27.27%)	8 (18.2%)	20 (8.4%)	14 (5.20)	3 (2.77%)
Marker chromosome	1 (0.3%)			1 (1.72%)		
Isochromosome	4 (1.5%)					1 (0.92%)

mosome. A large scale study is required to evaluate these abnormalities. Detailed analysis is described in discussion section.

DISCUSSION

Several studies have been carried out world-wide to determine the frequency of chromosomal abnormalities in PA. However, no such study has been performed in Pakistan so far.

Previous studies have reported a wide range of frequencies of chromosomal abnormalities, from 15.9% to 63.3% for primary amenorrhea[14,15].

Our result i.e. (18.5%), abnormal karyotype was in accordance with the results obtained by Safaei *et al*[11] showing 15 (20.4%) and Farnaz *et al*[16] (24.44%) chromosomal abnormality. However other studies such as Kong *et al* (58.8%)[17] and Butnariu *et al* (54.56%)[18] showed much higher frequencies of chromosomal abnormalities. Tahir et al showed (35.19) 13% abnormal karyotype[19].

One of our patient were found to have 46X with del Xp (p1.1). It is postulated that genes responsible for gonadal function is located in this region. Any disruption of this area leads to primary amenorrhea and all the other features of Turners syndrome. This abnormality is very rare and is not reported in other studies as shown in table-II. But a relatively similar abnormality in one patient was reported by Tahir et al i.e.Xq13-q26 in which patient had PA but no feature of Turner syndrome[19].

Male karyotype is also reported to be low in our study (3.0%) as compared to studies of Safaei *et al* (27.27%)[11] andFarnaz *et al* (18.2%)[16] but our results were similar to that of Tahir *et al*, revealing (2.77%) 13 of (XY) male karyotype[19]. XY individuals may have female genital organs as mullarian inhibiting factor and testosterone is not produced. Gonadal tumors may occur in such cases. Moreover, patients with male karyotype may also have ambiguous genitalia which is detected and diagnosed in early years of life. Relatively less number of such patients report with complaints of PA.

Marker chromosome and Isochromosome represents only a small percentage of chromosomal abnormalities[19,20]. To know exact significance of these chromosomes much larger scale studies are required.

These differences may be due to a wide variation of patients as they belong to different ethnic origins. Many patients with Turner and other syndromes do not report to hospital facility due to social pressures. Even girls get married without menstruating for once. We have diagnosed a case of 33 year old individual, married with male karyotype and another female with XO at the age of 35 years.

CONCLUSION

Frequency of cytogenetic abnormalities was found slightly lower in our population. One important reason is lack of awareness due to which less number of patients is referred for evaluation. Cytogenetic studies help to establish mode of inheritance and risk of recurrence in family which is useful in prevention and genetic counseling of family.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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