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Research Article

Cytogenetic Investigation of North Indian Patients with Primary Amenorrhea

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Abstract

Amenorrhea is seen as the sixth major reason for female infertility affecting 2-5% of the females in their childbearing age. Also, 2-5% of girls in their adolescent age are presented with PA (Primary Amenorrhea). PA is a symptom resulting from different cases like regulatory disorders in hypothalamus & pituitary glands or utero-vaginal deformations and affects the physiological as well as the physical well-being of the patient. This retrospective study was intended to analyze the incidence of chromosomal anomalies in North Indian patients with PA.

Materials and Methods: 168 PA cases were assessed by cytogenetic analysis and the results were further compared with other studies.

Results: Chromosomal abnormalities were observed in 14(8.33%) cases. The other 154 cases (91.1%) demonstrated a normal karyotype.

Conclusion: This study throws light on the significance of cytogenetic analysis in understanding the etiology concerning amenorrhea & in establishing a better genotype-phenotype correlation.

Keywords: Amenorrhea, Chromosomal Abnormalities, Isochromosome

Introduction

In a female of reproductive age, amenorrhea is characterized by lack of menstrual flow. It is most observed during pregnancy. The absence of menstrual flow is a normal feature in pre-pubertal and post-menopausal females. Amenorrhea can be classified primarily in two ways - Primary and Secondary Amenorrhea [1]. When menstrual periods fail to occur by 16 years in a female, the condition is recognized as Primary Amenorrhea (PA) [2]. Pubertal changes occur before the initiation of menstrual period (Menarche) and females who have attained the age 14 but have not encountered menarche, plus fail to show any indication for secondary sexual characteristics (like thelarche and pubarche), thus exhibit no signs for beginning puberty and are considered to have PA [3]. Secondary amenorrhea (SA) is described as the halting of menses in a woman with an established menstrual cycle [4]. It is characterized by the lack of menstrual flow for three months in a female demonstrating a normal menstrual cycle history, or for a woman with previously demonstrated irregular cycles, this ceasing of menstrual flow is for nine months [5]. Mullerian agenesis, vaginal obstruction, vaginal atresia, imperforate hymen (hymen lacking an opening), hypothalamic regulatory disorders, gonadal dysgenesis, and intersex conditions can contribute to PA [6]. Abnormal chromosomes lead to gonadal disorders which constitute about 50 percent of the PA incidences [7,8]. The chromosomal anomalies can be numerical (45, XO) or delicate structural alterations and deletions resulting in abnormally large or small chromosomes, or mosaicism (XO/XX and XO/XXX) [9-11]. Sex reversal cases in which the patient demonstrating female phenotype possesses a 45, XY karyotype is also observed in PA. Several researchers have conducted studies to ascertain the role played by these chromosomal abnormalities in females with PA. Cytogenetic investigation enables to get valuable insights about PA by providing a genome-wide picture of the chromosomes present in an individual. The current retrospective study was intended to evaluate the incidence of chromosomal anomalies in PA cases and the frequency of the same was compared with other studies.

Materials and Methods

This retrospective study was directed to determine the prevalence and incidence of chromosomal abnormalities in 168 PA cases who were referred to Pediatrics Research and Genetics Laboratory, Department of Pediatrics (Genetics Division) (Maulana Azad Medical College and Lok Nayak Hospital, New Delhi) for cytogenetic assessment from hospitals all over New Delhi. The study was directed over a period of 7 years (2010-2017).

Sampling and Karyotyping

For cytogenetic assessment, the standard protocol was followed which included first collecting 0.5 ml peripheral blood sample and then storing it in a heparinized vial [12]. Lymphocytes were cultured at 37°C for 72 hours inside an incubator in the culture medium. Following this, Colcemid was added for metaphase harvesting. Hypotonic treatment (1hr) was carried out which was followed by fixation by (3:1) methanol-acetic acid mixture. The cytogenetic assessment was carried out with respect to the directions of the International System for Human Genetics Nomenclature (ISCN, 2013) [13]. G banding involving trypsin and Giemsa (GTG Banding) was done to determine karyotypes (350 Band resolution) using Cytovision version 3.9 software and minimum 30 well banded and well-spread metaphases were analyzed [14].

Statistical Analysis

For data analysis, standard descriptive statistics were used. For meta-analysis, Comprehensive meta-analysis software was used [15].

Results

This study assessed 168 PA cases having a mean age of 21.4 years ranging from 14 to 29 years (SD=5.63). 14 cases (8.33%) out of the 168 cases investigated were found having chromosomal abnormalities. Out of these 14 cases, 10 demonstrated Mosaicism (45, XO/46, XX), 2 demonstrated Turner Syndrome (45, XO) and 2 demonstrated isochromosome (46, X,i(Xq)). The rest 154 cases demonstrated a normal karyotype (46, XX). Cytogenetic results have been tabulated below in Table-1.

Table 1: Cytogenetic results

SNo.	Cytogenetic result	No of cases observed	Frequency
1.	45,XO/46,XX	10	5.9%
2.	45,XO	2	1.19%
3.	46,X,iX(q)	2	1.19%
4.	46,XX	154	91.1%

Discussion

In any PA case, early diagnosis and understanding the etiology concerning amenorrhea is of primary importance to further enable effective counselling for future fertility possibilities. Cytogenetic assessment becomes mandatory to achieve the same. Different studies by researchers were directed to ascertain the prevalence of chromosomal anomalies in PA patients. The incidence of these chromosomal abnormalities has varied greatly. Our study was compared with studies conducted elsewhere and the results have been summarized in Table 2 and Table 3.

Table 2: Comparison with other studies

Other Studies	Total number of PA cases examined	Frequency of chromosomal abnormalities
Korgoankar et al (2018)[16]	490	24.7%
Kalavathi et al (2017)[17]	852	25.82%
Gosh et al (2017)[18]	150	23.9%
Ten et al (1990)[19]	117	31%
Vijayalakshmi et al (2017) [20]	140	27.8%
Tahir M. Malla et al (2016) [21]	108	35.18%
Farnaz Mohajertehran et al (2013)[22]	180	24.45%
Present study (2018)	168	8.33%

Forest plot of eight studies with estimates of chromosomal abnormality rates in PA cases. (95% Confidence Interval)

Korgaonkar et al. used Fluorescent in situ hybridization technique (FISH) and conventional cytogenetic techniques to evaluate the cytogenetic & clinical profile of 490 PA cases [16]. Out of the 490 cases they investigated, 121 demonstrated chromosomal abnormalities. Kalavathi et al. used GTG Banding to evaluate the prevalence of chromosomal anomalies in 852 cases of PA and found 25.82% cases to demonstrate chromosomal abnormalities (numerical, structural aberrations such as deletions, translocations) [17]. The latest study by Gosh et al. investigating patients with PA in Eastern India concluded that 23.9% of patients had chromosomal abnormalities, emphasizing the significance of cytogenetic investigation in PA [18]. Another study conducted by Ten et al. comprising of 117 females with PA, demonstrated that 36 cases (31%) out of 117, had structural or numerical abnormalities [19]. 27.8% cases out of the 140 cases evaluated by Vijayalakshmi et al. were marked with chromosomal abnormalities [20]. A study conducted by Tahir M. Malla et al. in 2016 on 108 females, suggested that 31.58% cases evaluated had chromosomal anomalies [21]. Farnaz Mohajerterhan et al. carried out chromosomal analysis on 180 PA cases and the karyotyping results demonstrated 44 cases with chromosomal abnormalities [22]. In the present study, 168 PA cases were analyzed, out of which 14(8.33%) revealed abnormal constitution of chromosomes and rest 154 demonstrated a normal 46, XX karyotype. The incidence of chromosomal anomalies can vary from 6.66-56.22% (6.66% was demonstrated by Joseph et al. in 1989) [23,24].

In our study, complete monosomy X(45,XO), Mosaic monosomy X(45,XO/46,XX) and isochromosomes were observed. The published reports have proved Turner' Syndrome or Mosaic monosomy X as the chief cause of PA[20]. Our study also revealed two females with 46,X,iX(q) karyotype. Structural abnormalities that lead to PA are commonly isochromosomes of q arm of X. X(q) appears to be a metacentric chromosome with structurally identical arms and same genes. Females with 46,X,iX(q) karyotype are observed to have streak gonads. Additionally, complete, and partial ovarian failure has been observed in iX(q) individuals. There is almost total lack of gonadal development in females with a 46,X,iX(q) karyotype.

Table 3: Statistics for each study calculated by using Comprehensive Meta-analysis software

Study Name	Event rate	Lower Limit	Upper Limit	Z-value	p-Value
Korgoankar et al(2018)	0.247	0.211	0.287	-10.644	0.000
Kalavathi et al(2017)	0.279	0.211	0.358	-5.047	0.000
Gosh et al(2017)	0.258	0.230	0.289	-13.481	0.000
Ten et al(1990)	0.308	0.231	0.397	-4.084	0.000
Vijayalakshmi et al(2017)	0.279	0.211	0.358	-5.047	0.000
Tahir M. Malla et al(2016)	0.352	0.268	0.446	-3.032	0.002
Farnaz Mohajertehran et al(2013)	0.244	0.187	0.312	-6.507	0.000
Present study(2018)	0.083	0.050	0.136	-8.590	0.000
	0.257	0.239	0.276	-21.38	0.000

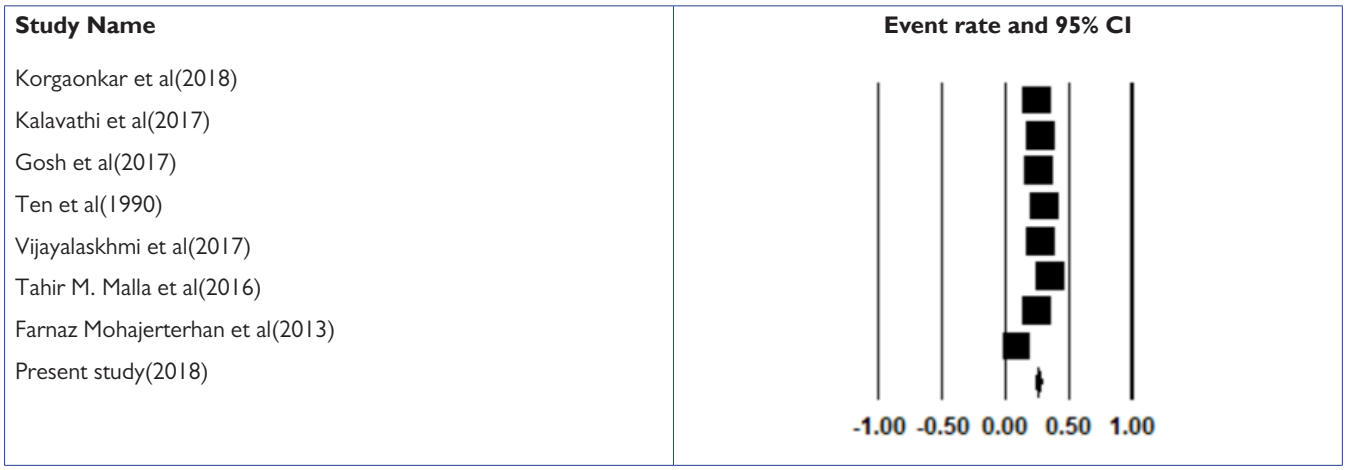

Figure 1: Forest plot of eight studies with estimates of chromosomal abnormality rates in PA cases. (95% Confidence Interval)

Conclusion

In this study, we have observed 8.33% cases with chromosomal abnormalities, thus, making them a plausible cause for PA. Cytogenetic assessment of PA cases becomes mandatory to enable precise diagnosis after the non-genetic sources have been excluded. It becomes a necessity to enable further counseling and developing an adequate treatment for the patients. If these chromosomal aberrations are characterized precisely, they can lead to recognition of novel genes associated with PA, enabling a better understanding of the mechanism involved.

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