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# Clinical and Cytogenetic profile of 490 cases of Primary amenorrhea

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**Abstract**
*Primary amenorrhoea (PA) is one of the common reproductive disorder affecting females.PA is characterised by the absence of menarche in the reproductive age group in females and some cases with absence of reproductive organs. The study was carried out in large cohort (N=490) of subjects with PA to identify genetic, anatomical and hormonal factors responsible for PA. Conventional cytogenetic and FISH (fluorescence in situ hybridization) technique were applied to detect the chromosomal abnormalities. The chromosomal abnormalities were detected in total 121 (24.7%) females. In our cohort numerical chromosomal aberrations frequency was found to be high (43.8%) as compared to structural abnormalities (26.4%). The 46, XY karyotype was identified in 29.8% patients. The anatomical abnormalities including absences of ovaries (51.2%), streak ovaries (35.6%), hypoplastic uterus (59.6%), absent uterus (22.3%) were also detected and correlated with genetic abnormalities in this cohort. FSH (follicular stimulating hormone) level were also found to be high in these patients. Our study suggests that genetic investigation are important for further management of the disease.*

**Keywords:** *Primary amenorrhea, Cytogenetics, Chromosomal abnormalities, Karyotype, Secondary Sexual Characters, Follicular stimulating hormone (FSH), Ultrasonography (USG).*

## Introduction

Primary amenorrhea (PA) is defined as no menstruation by age 14 in the absence of growth or development of secondary sexual characters, no menstruation by age 16 regardless of the presence of normal growth and development with the appearance of secondary sexual characters.[1] The world health organization (WHO) has estimated 15 % of the population as being infertile and amenorrhea as the sixth largest major cause of female infertility.[2] Among the general population amenorrhea seemed to have affected 2-5% of all women of child bearing age.[3]. The anatomical abnormalities of uterus, ovaries have been associated with PA. The chromosomal abnormalities play an important role in diagnosis of PA. Overall it is estimated that endocrine disorders account for approximately 40 % of the

causes of PA, with the remaining 60 % having developmental (genetic or structural) origins. Frequency of chromosomal abnormalities in primary amenorrhea ranges from 16% to 64%. [4]In this study we have analysed the types of chromosomal abnormalities seen in primary amenorrhea and correlated different clinical and hormonal factors with these abnormalities.

**Material and Methods**

Four hundred and ninety (490) cases of primary amenorrhea were studied for cytogenetic analysis during the period of 2005 to 2015. The age group of subjects ranging from 12 to 36 years and mean age was 18.77 ±5.56 years. The clinical details like age, height, secondary sexual character, hormone profile and USG findings were recorded in the case record sheet. The study protocols were approved by Institutional Ethics Committee.

**Cytogenetics**

Peripheral blood cultures were set up at $37^0$C for 72 hrs according to standard procedure. [5] The cultures were stimulated with phytohaemag-glutinin (PHA) arrested with colchicine (50 ug/ml) and treated with hypotonic solution (KCL-0.56g/100 ml). The cells were fixed in carnoy's solution (Methanol: Glacial acetic acid; 3:1). The chromosomal preparations obtained were subjected to GTG banding.[6] At least 30 to 50 metaphases depending upon chromosomal abnormalities were scored and karyotype (at 400 band resolution) according to International System Of Chromosome Nomenclature 2016(ISCN 2016).[7] Applied Spectral Imaging software system (Inc. Carlsbad, USA) interfaced with Nikon 90i microscope (Japan) were used for analysis.Fluorescence in situ hybridization (FISH) was done according to standard procedure using centromeric (CEP) and whole chromosome painting (WCP) probes for X and Y chromosomes (Vysis, Abbott Molecular Inc., Des Plaines, IL).

**Results**

Among 490 PA cases studied, the chromosomal abnormality was detected in 121 (24.7 %) cases. In our series the frequency of the numerical chromosomal changes (43.8%) found to be high compared to structural aberrations (26.4%) (Table 1). The 46,XY karyotype was identified in (29.8%) (Fig.1). The age of the patients at presentation ranged between 12-36 years and the mean age of presentation in different categories of PA with cytogenetic abnormality is shown in figure 1. Distribution of cytogenetic abnormality, mean age of presentation and height is presented in table 2. It was observed that 45.4% cases of PA with cytogenetic abnormality had height less than 150 cm and 54.6 % cases has height more than 150 cm and above. Secondary sexual characters like pubic hairs and axillary hairs were correlated with cytogenetic abnormality (Table 3).Axillary hairs were well developed in 33.9%, absent in 50.4% and sparse in 15.7% cases and pubic hairs were well developed in 33%, absent in 49.6% and sparse in 17.4% cytogenetically abnormal cases of PA. Tanners staging of breast development and cytogenetic abnormality was presented in table 4. The most frequent stage of breast development was stage 2 (35.6%) and other stages in decreasing frequency were: stage 3 (24%), stage1 (19%), stage 4 (12.4%) and stage 5 (9%). Correlation of cytogenetic abnormality, FSH level and ultrasonography findings in PA cases were shown in table 5.Mean FSH levels in different chromosomal abnormalities in PA is depicted in figure 3. In females with abnormal karyotype a significantly high level of FSH were detected. USG findings showed that uterus was normal in 18.1%, hypoplastic in 59.6% and absent in 22.3% cases and ovaries were normal in 13.2%, absent in 51.2% and streak in 35.6% cases.

**Table 1:** Incidence of chromosomal abnormality in PA

| S.No. | Cytogenetic category | | | Karyotype | Number | Percentage |
|---|---|---|---|---|---|---|
| 1 | Normal | | | 46,XX | 369 | 75.3% |
| 2 | Chromosomal abnormality | | | | 121 | 24.7% |
| | 1 | Numerical abnormalities | | | 53 | 43.8% |
| | | a. | Pure Turner | (45,X) | 28 | 23.2 |
| | | b. | Trisomy X | 47,XXX | 1 | 0.8 |
| | | Mosaicism of X | | | 24 | 19.8 |
| | | | | 45,X/46,XX | 17 | 14.0 |
| | | | | 45,X/46,XY | 3 | 2.4 |
| | | | | 46,XX/47,XXX | 3 | 2.4 |
| | | | | 45,X/46,XX/47,XXX | 1 | 0.8 |
| | 2 | Structural abnormalities | | | 32 | 26.4 |
| | | a. | Deletion Xq | 46,X,del(Xq) | 10 | 8.3 |
| | | b. | Iso-chromosome | 46,X,iso(Xq) | 9 | 7.4 |
| | | c. | Translocation | 46,X,t(X;A) | 4 | 3.3 |
| | | d. | Idic | 46,X,idic(X)p | 1 | 0.8 |
| | | e. | Marker chromosome | 46,X+marker | 4 | 3.3 |
| | | f. | Inversion 9 | 46,XX,inv(9) | 4 | 3.3 |
| | 3 | Male karyotype | | 46,XY | 36 | 29.8 |

**Table 2:** Distribution of cytogenetic abnormality, age and height in primary amenorrhea cases

| Sr. no. | Cytogenetic abnormality | Age in years (Mean±SD) | Height (cm) | |
|---|---|---|---|---|
| | | | < 150 | 150 and above |
| 1 | 45,X (28) | 17.04 ±3.43 | 25 (89.3%) | 3(10.7%) |
| 2 | Mosaicism X (24) | 19.71± 6.54 | 7(29.2% | 17(70.8%) |
| 3 | Trisomy X (1) | 17±0.00 | 00 | 1(100%) |
| 4 | Structural abnormality (32) | 19.84 ± 4.57 | 23(71.9%) | 9(28.1%) |
| 5 | Male karyotype (36) | 20.28±7.72 | 00 | 36(100%) |
| Total | | | 55(45.4%) | 66(54.6%) |

**Table 3:** Distribution of cytogenetic abnormality and secondary sexual characters in PA

| Sr.No. | Cytogenetic abnormality | Axillary hairs | | | Pubic hairs | | |
|---|---|---|---|---|---|---|---|
| | | Present | Absent | Sparse | Present | Absent | Sparse |
| 1 | 45,X (28) | 0 | 28 (100%) | 0 | 0 | 28(100%) | 0 |
| 2 | Mosaicism X (24) | 13 (54.2%) | 9(37.5%) | 2(8.3%) | 13(54.2%) | 9(37.5%) | 2(8.3%) |
| 3 | Trisomy X (1) | 0 | 0 | 1(100%) | 0 | 0 | 1(100%) |
| 4 | Structural abnormality (32) | 12(37.5%) | 9(28.1%) | 11(34.4%) | 12(37.5%) | 8(25%) | 12(37.5%) |
| 5 | Male karyotype (36) | 16(44.4%) | 15(41.7%) | 5(16.6%) | 15(41.7%) | 15(41.7%) | 6(100%) |
| Total 121 | | 41(33.9%) | 61(50.4%) | 19(15.7%) | 40(33%) | 60(49.6%) | 21(17.4%) |

**Table 4:** Distribution of cytogenetic abnormality, breast development (Tanner staging) in PA

| S.No. | Cytogenetic abnormality | | Tanner stage | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | Pure turner (45,X) | No. | 10 | 12 | 6 | 0 | 0 | 28 |
| | | % | 35.7% | 42.9% | 21.4% | 0.0% | 0.0% | 100.0% |
| 2 | Male Karyotype (46,XY) | No. | 4 | 12 | 11 | 6 | 3 | 36 |
| | | % | 11.1% | 33.3% | 30.6% | 16.7% | 8.3% | 100.0% |
| 3 | Mosaicism of X | No. | 3 | 10 | 4 | 6 | 1 | 24 |
| | | % | 12.5% | 41.7% | 16.7% | 25.0% | 4.1% | 100.0% |
| 4 | Structural Abnormalities | No. | 6 | 9 | 8 | 2 | 7 | 32 |
| | | % | 18.8% | 28.1% | 25.0% | 6.2% | 21.9% | 100.0% |
| 5 | Trisomy X | No | 0 | 0 | 0 | 1 | 0 | 1 |
| | | % | 0.0% | 0.0% | 0.0% | 100% | | 100% |
| | Total | No. | 23 | 43 | 29 | 15 | 11 | 122 |
| | | % | 19% | 35.6% | 24% | 12.4% | 9% | 100% |

**Table 5**: Distribution of cytogenetic abnormality, FSH level and USG findings in PA

| Sr. No. | Cytogenetic abnormality | FSH M±SD | USG finding (Uterus) | | | USG finding (Ovary) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Normal | Hypoplastic | Absent | Normal | Absent | Streak |
| 1 | 45,X (28) | 90.75±60.72 | 1(3.6%) | 21(75%) | 6(21.4%) | 0 | 20(71.4%) | 8(28.6%) |
| 2 | Mosaicism X (24) | 61.01±51.86 | 6(25%) | 16(66.7%) | 2(8.3%) | 4(16.7%) | 8(33.4%) | 12(50%) |
| 3 | Trisomy X (1) | 4.46±0.00 | 0 | 1 | 0 | 0 | 0 | 1 |
| 4 | Structural abnormality(32) | 56.51±41.02 | 11(34.4%) | 20(62.5%) | 1(3.1%) | 11(34.4%) | 7(21.9%) | 14(43.8%) |
| 5 | Male karyotype(36) | 51.18±33.76 | 4(11.1%) | 14(38.9%) | 18(50%) | 1(2.8%) | 27(75%) | 8(22.2%) |
| Total 121 | | | 22(18.1%) | 72(59.6%) | 27(22.3%) | 16(13.2%) | 62(51.2%) | 43(35.6%) |

**Table 6:** Scenario of genetic anomalies reported in earlier studies of PA cases worldwide.

| Sr. No | Study | Study population | Study period/ Year | No. of cases | Normal Karyotype | Abnormal karyotype |
|---|---|---|---|---|---|---|
| 1 | Van Niekerk et al [8] | South Africa | 1978 | 77 | 56(72.7%) | 21(27.3%) |
| 2 | Ten et al [9] | Malaysia | 1990 | 117 | 81(69.2%) | 36(30.8%) |
| 3 | Roy et al [10] | India | 1995 | 60 | 22(36.5%) | 38(63.5%) |
| 4 | Temocin et al [11] | Turkey | 1997 | 68 | 50(73.5%) | 18(26.5%) |
| 5 | Lakshmi Kalpana et al [12] | India | 1998 | 70 | 50(71.43%) | 20(28.57%) |
| 6 | Mondel et al [13] | India | 2002 | 72 | 48(66.67%) | 24(33.33%) |
| 7 | Wong et al [4] | HongKong | 1991-2002 | 237 | 179(75.5%) | 58(24.5%) |
| 8 | Cortes et al[14] | Mexico | 1995-2003 | 187 | 109(58.28%) | 78(41.72%) |
| 9 | Rajangam et al [1] | India | 1973-2005 | 620 | 458(73.87%) | 162(26.13%) |
| 10 | Hariharan et al [15] | India | 2003-2006 | 51 | 25(49.2%) | 26(50.8%) |
| 11 | Vijayalaksmi et al [16] | India | 1998-2006 | 140 | 101(72.15%) | 39(27.85%) |
| 12 | NaushabaRizwan et al [7] | Pakistan | 2006-2007 | 19 | 14(73.68%) | 5(26.32%) |
| 13 | Safei et al[18] | Iran | 2005-2008 | 220 | 176(80%) | 44(20%) |
| 14 | Tanmahasamut et al[19] | Thialand | 1992-2009 | 295 | 236(80%) | 59(20%) |
| 15 | Faeza EL Dahoty[20] | Egypt | 2008-2010 | 223 | 177(79.37%) | 46(20.63%) |
| 16 | Merin T et al [21] | India | 2006-2012 | 246 | 210(85.36%) | 36(14.64%) |
| 17 | Sapna Amin et al[22] | India | 2006-2012 | 98 | 78(79.5%) | 20(20.5%) |
| 18 | Present study | India | 2000-2015 | 490 | 369(75.3%) | 121(24.7%) |

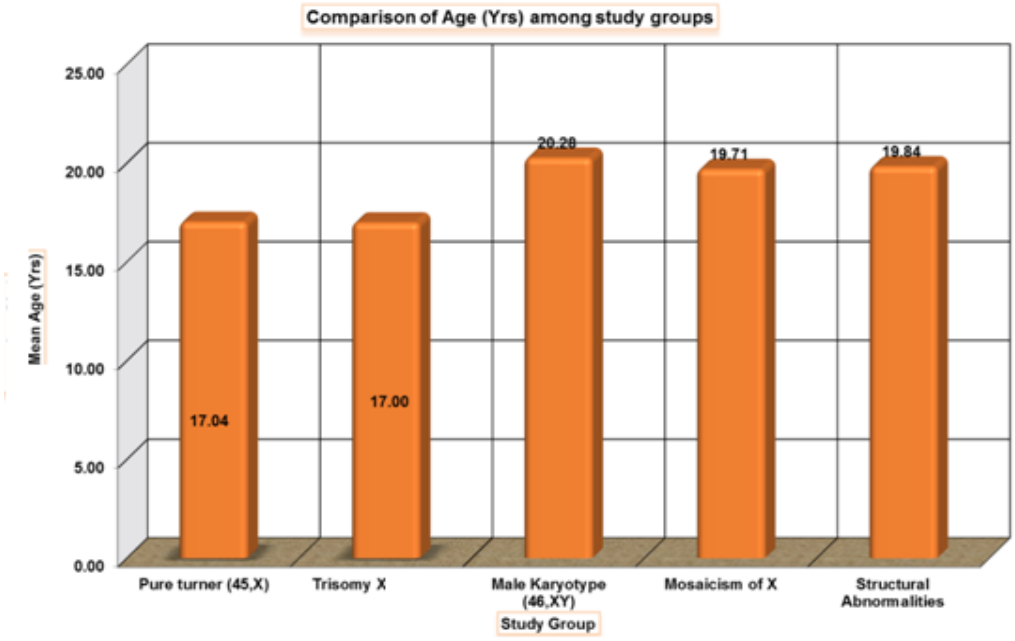


**Figure 1**: Age wise distribution of chromosome abnormalities in PA.

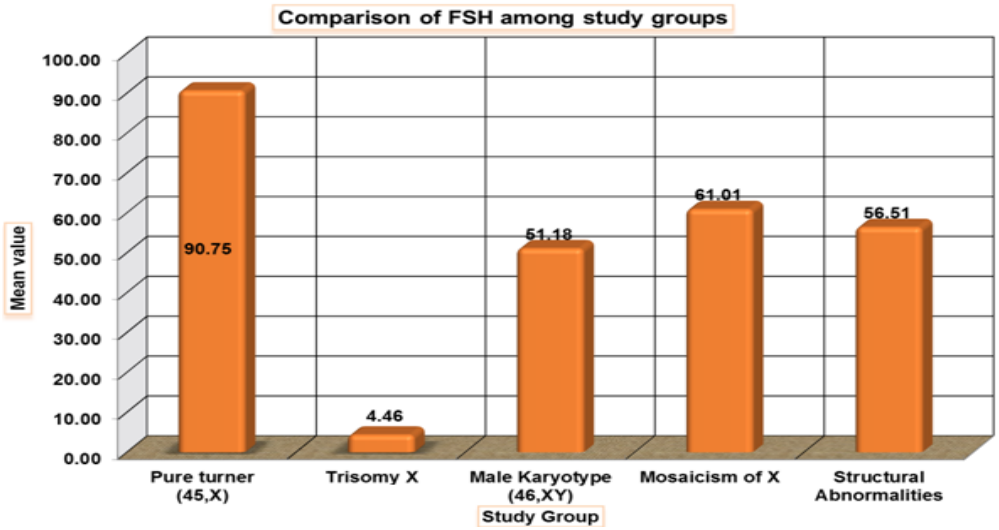


**Figure 2:** Mean FSH levels in different groups of chromosomal abnormalities.

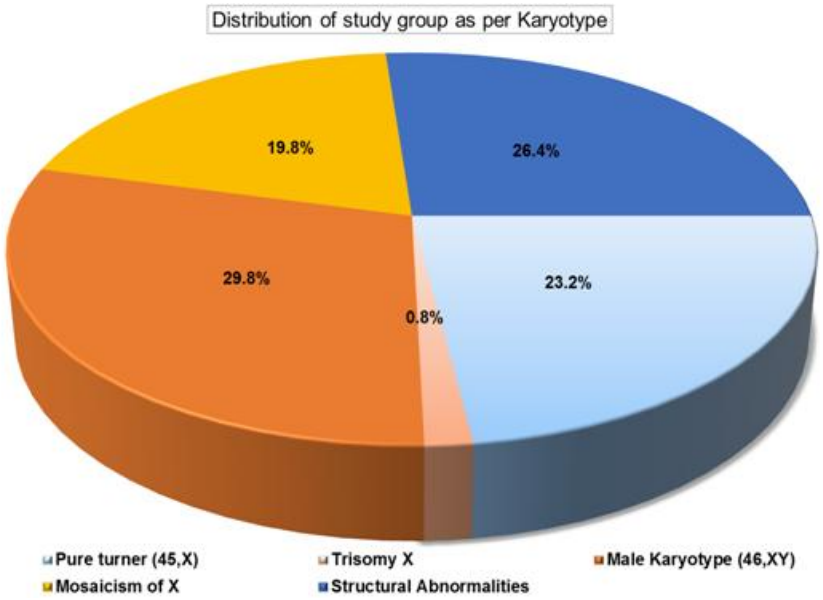


**Figure 3:** Frequency and distribution of chromosomal abnormalities in PA

## Discussion

The PA occurs due to several factors including hormonal imbalance, anatomical abnormalities, genetic factors and environmental factors. However chromosomal aberrations especially sex chromosome aberrations plays an important role in PA, hence knowledge of cytogenetics is essential for further management of these cases. The chromosomal aberration frequency reported to be ranging from 14 to 60%.[3-4,8-23] In our study, chromosome aberrations were identified in 121 (24.7%) out of 490 PA cases studied (Table 1). The frequency of chromosome aberrations of our study is similar to studies reported from different parts of the world (Table 6). In our cohort the numerical chromosomal aberrations including monosomy X and X chromosome mosaicism were more (43.8%) compared to structural aberrations of X chromosome (26.4%).Because the advantages of FISH we were able to identify X chromosome abnormalities in 19.8% mosaic cases. Overall numerical abnormality of X chromosome is common in PA cases. However, structural abnormalities of X chromosome such as Xq deletions, isoXq, isodicentric X equally associated with PA. The structural changes in Xq region is important as the genes located on these regions essential for gonadal function and structural abnormalities in Xq leads to the gonadal dysgenesis.[20, 23]

Interesting observation in our cohort is that a high frequency (29.8%) of 46,XY karyotype in females with PA.Those females who present as PA with karyotype 46,XY are phenotypically female since the abnormal gonadal tissue in these cases fails to produce Mullerian inhibiting factor and testosterone. Gonadal tumours occur in up to 25% of women with a Y chromosome, unlike complete androgen sensitivity syndrome; these gonads do not secrete hormones and should be removed at the time of diagnosis. [8] As the incidence of male karyotype in this study is high as compared to previous reported studies (16%). [4, 9-23], the presence of Y chromosome should be confirmed by FISH. At the same time these cases needs to be further assessed for any mutation of SRY gene, SF1 gene and the other genes which are responsible for male karyotype in phenotypic female with PA. [24, 25] These cases should be correlate with genetic investigation for appropriate genetic counselling. The clinical evaluation including height and secondary sexual characters is important in diagnosis of PA. The short stature and poor development of secondary sexual characters may be indication for the diagnosis of PA as in our study the chromosomally abnormal PA cases had short stature (<150cm) and poor development of secondary sexual characters (Table 2, 3). In the present study out of 121 cases of PA with chromosomal abnormality, 95 (78.6%) cases had stages 1, 2, 3 of breast development, which suggests that under developed breast might be one of the clinical features in PA (Table 4). In our series hypoplastic uterus(59.6%), absence of uterus(22.3%) , absence of ovaries(51.2%) and streak gonads(35.6%) were found to be are major anatomical abnormalities and similar abnormalities also have been reported in earlier studies (Table 5).[24,27]

In conclusion the women with the absence of menstruation and secondary sexual characters should be investigated for chromosomal abnormality along with the routine hormonal and radiological investigations. More emphasis should be given to cytogenetic investigations as the clinical signs and symptoms are found to be variable in these cases.After exclusion of non-genetic causes, patients with PA should receive prompt referral for genetic and molecular study. Genetic counseling should include the risk of premature menopause for patients with Turner's syndrome, possibility of pregnancy in cases with X mosaicism and the use of hormone replacement therapy, the risk of gonadal malignancy for patients with XY GD and the possibility of infertility in patients with other chromosomal aberrations.

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**Conflict of Interest:** Nil

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