The primary amenorrhea (PA) is defined as absence of menstruation by the age of 16 years in the presence of normal secondary sexual characters or 14 years in the absence of other evidence of puberty. The incidence of PA is < 1% (WHO), the sixth largest cause of female infertility. The etiological factors are endocrine disorders 40% and developmental (genetic or structural) 60%. The frequency of chromosomal abnormalities in PA is 16% to 78%. In PA, 46,XY disorder of sex development (DSD) is seen in 14% cases, out of which androgen insensitivity syndrome (AIS) is seen in (9%) cases and gonadal dysgenesis in 5%. Disorder of sex development is a group of congenital condition in which the development of chromosomal, gonadal, or anatomical sex is atypical.

**Methods**

1. **Karyotyping** - Chromosome preparation was done using standard protocol followed by GTG banding. Minimum 30 metaphases were analysed using 90X microscope (Nikon Japan) and were karyotyped according to the ISCN 2009.
2. Fluorescence In Situ Hybridization (FISH) was carried out using centromeric probes for X and Y chromosome as per the standard protocol and images were captured and analysed using ASI software.
3. **Molecular study**
   - Genomic DNA was extracted from peripheral blood using human blood DNA extraction kit (Qiagen) as per the standard protocol.
   - Conventional PCR was performed for amplification of SRY, AR, SRD5a2 and SF1 genes and direct sequencing was done using ABI -3130 Genetic Analyzer for the amplified products.
   - Sequence analysis was done using Chromas Lite software and nucleotide sequences were compared with the wild type DNA sequences.

**Results**

1. **Endocrine factors** in cases of PA were found to be, anatomical (39.3%), genetic (30.8%) and endocrine (29.9%) and is shown in figure no.1
2. **External genitalia** were found to be ambiguous in 29% cases, testis was found to be absent in 58% cases, and gonads were found to be absent in 33% and testis in 46% cases and is presented in figure no.2.
3. **Sex chromosome analysis** using FISH was carried out in 45 cases and the result of SRY gene in 1 case and SRY 2 part is deleted in 1 case.
4. **SRY gene perturbation** in our study is 83.3% and perturbations of SRY gene as previously reported studies are seen in 10-15% of gonadal dysgenesis cases.
5. A novel mutation, c.81C>G and 11 bp deletion (c.82_92delGGAGACCTCCCGG) was identified in SRD5a2 gene exon 1. Chromatogram is shown in figure no.3
6. **The data on SRY, SRD5a2, AR and SF1 genes in XY females with PA is shown in table no.2**

**Conclusions**

In the present study, out of 341 cases of PA, 105 cases (30.8%) had chromosomal abnormalities and 46,XY DSD was found in 37 cases (35.23%). Early genetic investigation is found to be essential for the further management. SRY gene perturbation in this study is 8.33%. SRD5a2 gene mutation is 5%. AR gene and SF1 gene did not revealed any mutation in this study. As mutations were not detected in many of these patients in above mentioned candidate genes, other chromosomal loci (DX1, DH1, WT1, SOX9 etc.) should be investigated to find out molecular mechanism in these complex clinical situations. PA cases with DSD represents a group of various different conditions with wide phenotypic spectrum where molecular genetic diagnosis is found to be difficult. The newer technique like Next Generation Sequencing (NGS) will help to co-relate genotype with variable phenotype in these cases. After karyotyping, patients with PA should receive prompt referral for genetic and molecular study. Genetic counseling should include the risk of gonadal malignancy for patients with XY GD and the possibility of infertility.

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**References**