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Karyotyping Study in Primary Amenorrhoea

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ABSTRACT

Primary amenorrhoea, defined as the absence of menstruation by age 14 without secondary sexual characteristics or by age 16 with them, is a clinical symptom with diverse etiologies. **Objective:** To determine the frequency and types of chromosomal abnormalities in patients presenting with primary amenorrhoea and to evaluate the clinical significance of cytogenetic analysis in diagnosis and management. **Methods:** A 25 cases detailed assessment including clinical history, physical examination, hormonal profiling, ultrasonography, and chromosomal analysis was conducted on individuals presenting with primary amenorrhoea, in human genetic lab from B.J. MEDICAL COLLEGE, AHMEDABAD, GUJARAT. **Results:** Chromosomal abnormalities were identified in a significant proportion of cases, ranging between 15.9% and 63.3%, with Turner's syndrome, gonadal dysgenesis, and androgen insensitivity syndrome being the most frequently observed. Most anomalies involved numerical or structural abnormalities of the X chromosome. **Conclusion:** Cytogenetic evaluation is crucial in the diagnostic workup of primary amenorrhoea, aiding in accurate diagnosis, prognosis estimation, and guiding genetic counseling. Incorporating karyotyping into routine assessment enhances clinical decision-making and patient outcomes.

Key Words: *karyotyping, chromosome.*

INTRODUCTION :

Amenorrhea means absence of menstruation during puberty or later in life. Menstrual disorders are one of the most commonly seen gynaecological problems in adolescent girls, composing 75% of such problems worldwide[2]. World Health Organization had estimated that out of 15 % infertility in the human population, amenorrhoea stands as sixth largest major cause of female infertility. Among general population amenorrhoea affects 2% - 5% of all women in the child – bearing age[1]. Amenorrhea is only a symptom and not a disease entity. Amenorrhea can be physiological or pathological[2]. There are two types of amenorrhoea; they are primary amenorrhoea (PA) and secondary amenorrhoea (SA). Primary amenorrhoea is defined in two different groups. In the first group it is defined as the absence of menarche by age 14 years, with no development of secondary sexual characters. In the second group, it is defined as the absence of menarche by age 16 years with normal development of secondary sexual characters. Secondary amenorrhoea (SA) is clinically defined as the absence of menses for more than 3 cycle intervals or 6 consecutive months in previously menstruating women[2]. Menstruation is the endpoint of a series of events which begin in the cerebral cortex and hypothalamus and terminates at the uterine and ovarian tissues in the hypothalamic pituitaryovarian axis (HPO axis). Any break in this axis creates menstrual problems. Regular and spontaneous menstruation requires: a) an intact hypothalamic-pituitary-ovarian endocrine axis, b) an endometrium competent to respond to steroid hormone stimulation and c) an intact outflow tract from internal to external genitalia[2]. Pathological amenorrhea can be the result of genetic variations, systemic diseases, endocrinopathies, disturbance of the Hypothalamic Pituitary Ovarian axis, gynatresia, nutritional factors, drug usage, absence of uterus and vagina, endometritis, improper functioning of ovaries, mullerian agenesis, psychological factors and other rarer causes. Amenorrhoea is a normal clinical feature (physiological amenorrhoea) in prepubertal, pregnant, and postmenopauasal females. It also accounts for 20% of patients with infertility[3]. The genetic factors like single gene disorders, chromosomal abnormalities, or multifactorial disorders are frequent causes of amenorrhoea. Cytogenetic investigations have shown the importance of chromosomal abnormalities as a major cause of amenorrhea. Amenorrhea patients show a variety of chromosomal abnormalities like 45,X and sex reversals i.e., female phenotype with male chromosomal compliments and also other autosomal translocations. Sex chromosomal abnormalities as the cause of Primary amenorrhoea is well documented. These sex chromosomal abnormalities may be numerical with XO/XXX and structural showing Xp/q duplications or deletions, ring chromosomes, isochromosomes, and mosaics leading to Primary amenorrhoea[1]. The

physiology of menstruation and reproduction has a strong correlation with the expression of the X chromosome. Thus, the role of genetics in terms of diagnosis, risk assessment,and genetic counselling is significant. The genetic contribution to amenorrhea is studied both at the cellular and molecular level aiming at abnormalities in chromosomes and mutations in genes[3]. The percentage of chromosomal abnormalities varies from 15.9% to 63.3 % in Primary amenorrhoea. This wide variation is due to different sample size of selection group. Indications for genetic analysis includes developmental delay, advanced maternal age, fertility problems and family history of chromosomal abnormalities. Our study aimed to determine the frequency of chromosomal abnormalities and also to find out the most prevalent chromosomal anomalies present in Primary amenorrhoea. Karyotyping aids in confirmation of provisional diagnosis a better phenotype-genotype correlation to understand clinical heterogeneity and in genetic counselling[3].

Materials & Methods :

The present study includes observations of 25 cases diagnosed as Primary amenorrhoea from Civil Hospital, Ahmedabad, during the year of 2015. Subject's personal data including name, age, sex, onset and type of illness, relevant personal history, past history and family history were noted. In addition, vital statistics, brief clinical assessment, all routine and specific investigations regarding Primary amenorrhoea were also noted. Karyotyping study was done in all 25 cases.

Ethical Considerations

All blood sample were pre-exiting from collection at civil hospital , obs& gynec department, B.J. Medical College, Ahmedabad, with institutionalethical approval, ensuring respectful handling of human remains and adherence to ethical standards for anatomical research.

EXCLUSION CRITERIA:

- Patient refusing to give consent

Method: Blood samples of the patients were obtained in a heparinized container. The cultivation was done under all aseptic precautions on the same day of the aspiration. After an incubation period of 69.5hours at 37°C, the cell division was arrested atmetaphase stage byadding colchicine and after total 72 hours of incubation period the lymphocytes were harvested. sample was centrifuged to remove supernatant fluid at 3000 rpm for 10 minute. The hypotonic solution (KCl 0.075 M) was added at 37°C for 20 min to swell the cells, and the sample was treated thrice with chilled fixative (3:1 ratio of methanol: acetic acid). Finally, the slides were prepared for Karyotype study. All the slides were scanned for metaphase detection. The readings were then taken from each slide and noted. Thereafter, those slides showing metaphase with good morphology were selected and kept under non-humid dry wooden boxes for aging process. Approximately after 7 days of harvesting, Giemsa banding procedure was done using freshly prepared Trypsin-EDTA solution and Giemsa stain. The procedure protocols were followed according to the guidelines from the book Rooney and Czepulkowski, Human Cytogenetics: A practical approach. A band is defined as the part of chromosome, which is clearly distinguishable from its adjacent segments by appearing darker or lighter. The first banding method was quinacrine fluorescence method; developed in 1970, which was followed by various methods of Giemsastaining. A Giemsa dye mixture reveals identical patterns of dark and light bands along the chromosomes. This kind of technique is called G-Banding method and resulting bands are G bands where the dark bands contain mainly A-T rich DNA and the light bands are G-C rich DNA. About 30 metaphase plates were observed in each case and finally, a photograph was obtained from a good quality metaphase slide. The chromosomal findings were described according to the international system of Human Cytogenetic Nomenclatures and finally, Karyotypes were prepared using Automatic Karyotyping software. Correlation of chromosomal finding was done with similar studies done in past.

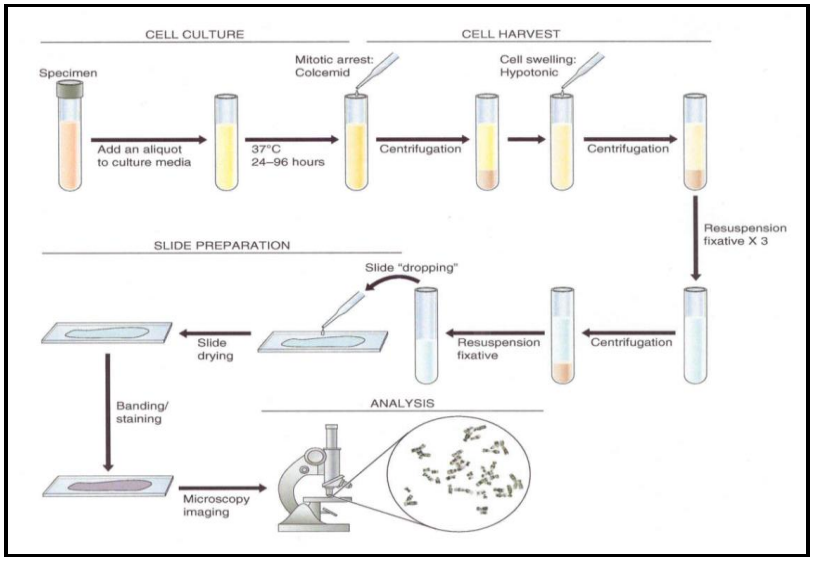

Figure 1: Karyotyping Procedure for slide preparation

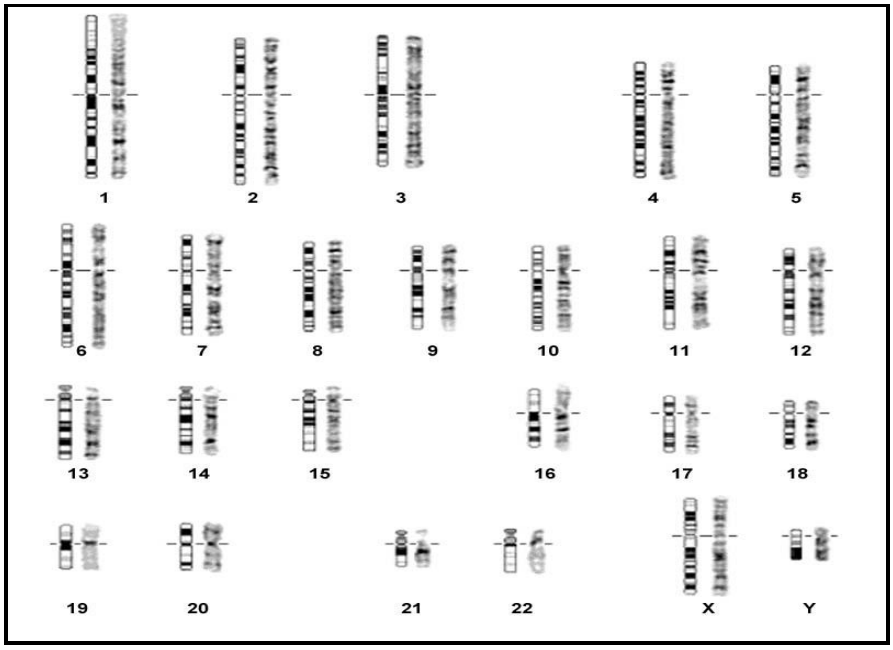

Figure 2: Ideogram

Observation & result : The study group included the patients who attended outdoor and indoor patient departmentsin obstetricand gynecology, civil hospital, Ahmedabad, during the period of year 2013 to 2015. Total 25 patients were studied, who were diagnosed clinically as "Primary amenorrhoea".

The age groups of the patients in the study were as follows:

Table-I

Showing Age distribution in primary amenorrhea patients:

Age (in years)	Primary amenorrhoea patients	
	No. of patients	Percentage(%)
11–12	4	16
13-14	7	28
15-16	8	32
17-18	3	12
19-20	1	4
21 and above	2	8

Above table shows that out of 25 patients studied, 4(16%) were in the age group of 11-12 years, 7(28%) in the age group of 13-14 years, 8(32%) in the age group of 15-16 years, 3(12%) in age group of 17-18 years, 1(4%) in the age group of 19-20 years and 2(8%) patients were above 21 years of age .

Table-II

Showing distribution of cases according to marital status in parimary amenorrhoea patients:

Status	Primary amenorrhoea patients	
	No. of patients	Percentage(%)
Married	2	08
Unmarried	23	92

Above table shows, out of 25 patients studied, 2(8%) were married and 23(92%) were unmarried.

Table-III

Showing Frequency of symptoms in primary amenorrhea patients:

Symptoms	Lump in abdomen	Pain in abdomen
Number of patients	5	11

The above table shows that out of all 25 patients who presented with primary amenorrhoea; 11 patients had pain in abdomen and 5 patients had lump in abdomen.

Table-IV

Showing Height in cms in primary amenorrhoea patients:

Height in cms.	140cms-145cms	146cms-150cms	151cms-155cms	156cms and above
Number of patients	2	14	9	-

Above table showsthat 14(56%) of patients was having height between 146-150 cms, 9 (36%) had height between 151-155 cms, and 2(8%) patients had height below 145 cms. 1 patient aged 12 years was having whose height was 141 cms, also presented with short stature, webbed neck and other turner like symptoms.

Table-V

Showing Weight in primary amenorrhoea patients:

Weight in Kg.	31-35kg	36-40kg	41-45kg	46-50kg	51 and above
Number of patients	4	5	9	5	2

The weight of the patients was found 41-45 kg in 9 patients, 36-40 kg and 46-50 kg in 5 patients each, 31-35 kg in 4 patients and 2 patient were having above 51 kg of weight.

Table-VI

Showing Secondary sex characters in primary amenorrhoea patients:

Secondary Sex characters	Not developed	Poorly developed	Normally developed
Number of patients	2	1	22

The above table shows that out of 25 patients,22 patients had normally developed secondary sex characters, 1 patient had poorly developed and in 2 patients there was absence of secondary sex characters.

Table-VII

Showing Congenital anomalies in primary amenorrhoea patients:

Congenital anomalies	Absent uterus	Bicornuate Uterus	Imperforate hymen	Blind vagina	Steak ovaries	Renal malformation
No. of patients	2	1	1	3	1	3

Out of 25 patients studied 7 patients went for diagnostic Laproscopy. Above table shows that out of 25 , 7 (28%) patients had congenital anomalies. Absent uterus found in 2 (8%), bicornuate uterus found in 1(4%), imperforate hymen found in 1(4%), blind vagina found in 3(12%), steak ovaries found in 1(4%) and renal malformation found in 3(12%).

Table-VIII

Showing cytogenetic findings in primary amenorrhoea patients:

Metaphase findings	Primary amenorrhoea patients	
	No. of patients	Percentage(%)
Numerical	1 (Turner)	4
Structural abnormality	1 [46,X,i(X)(q10)]	4
Normal Karyotype	23	92

Above table shows the cytogenetic finding in 25 cases, 23 (92%) cases were having normal karyotype, 1 (4%) case was having structural abnormality in the form of 46,Xi(X)(q10) and 1 (4%) case was having 45 X karyotype suggestive of turner syndrome.

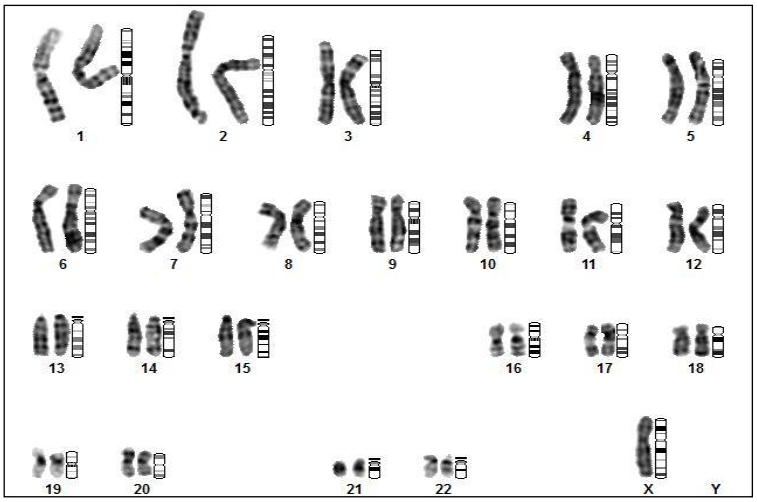

Fig.3. 12 year old female sample: Turner's syndrome 45,X

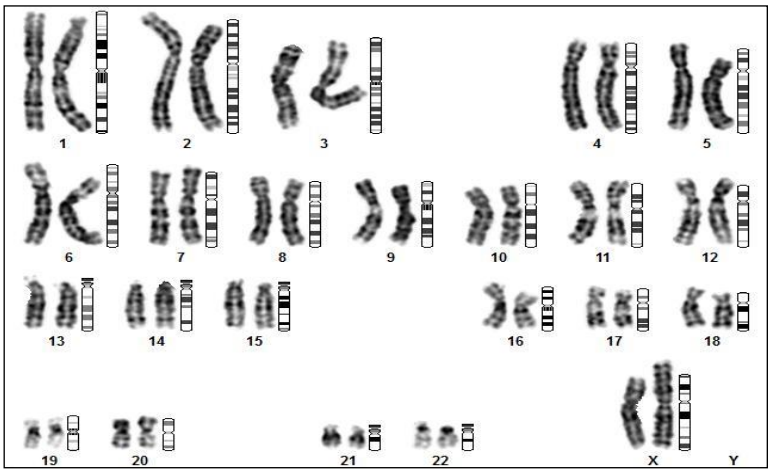

Fig. 4. 20year old female sample : Isochromosome 46,X,i(X)(q10)

Discussion:

Amenorrhoea is the most commonly seen gynaecological problem and is the 6th major cause of female infertility. Etiology of primary amenorrhoea includes hormonal imbalance, genital tract problems and chromosomal abnormalities. Inspite of great advances in knowledge of genital anomalies the differential diagnosis of various syndromes related to primary amenorrhoea are still remains an open problem. To aid in diagnosis in present study 25 cases of primary amenorrhoea were selected. Relevant history and clinical parameters were noted. Karyotyping study of all 25 cases was done to find out chromosomal abnormalities.

- Age distribution:

  In present study table-I shows age distribution of patients. It indicates that maximum number of patients were in the age group of 13-16 years, presented with primary amenorrhoea, only two patient was more than 20 years of age. Dr.Purandera studied 109 cases, In that study age variation was found between 15 -18 years in more than 70% of cases and 6% of patients were more than 25 years of age[4]. Rao studied 45 cases, In that study age variation was found between 14 – 17 years in more than 68 % of cases and 9% of patients were above 20 years of age[40].
- Marital status:

  It is found that Primary amenorrhea is one of the causative factors of disturbances in normal marital and social life of most of the patients. In Present study out of 25 cases, 2 patients were married, amongst them the duration of their married life was 6 months in 1 patients and 4 years in another patients. The remaining 23 Patients were unmarried.
- Patient presentation:

  In present study (Table III) failure of onset of menstruation was the chief complain of all the patients. Along with that some had associated complains like pain in abdomen, lump in abdomen etc. 11 patients had pain in abdomen and 5 presented with lump in abdomen. In present study, one patient was found having Turner's like features - short stature, shield chest and webbed neck. WS. Daniel. out of 20 patients recorded 1 patient with short stature, kyphosis, shield-like chest, underdeveloped secondary sexual characteristics and primary amenorrhea[6]. In Jacobs study out of 18 patients, 3 patients were presented with webbed neck, short stature and abdominal pain[7]. In Lyon study out of 18 patients, 5 patients presented with complain of primary infertility[8].

- Height and Weight:
  In present study Table IV and V14 (56%) patients were with 146cms-150cms of height and 2 (8%) patients were below 145 cms of height. The weight of 9 patients was 41-45 kg. and the weight of 2 patients was above 51 kg. 1 (4%) patient aged 12 years was 141 cms. tall and of 37kg. weight, also presented with short stature, webbed neck and other turner like symptoms.
  Oastler and Sutherlend found one case of obesity out of 30 studied cases, which was the causative feature of amenorrhea[9]. Wong MS, presented a case of 16 years old patient, having height 129 cms. and weight of 28 kg. with karyotyping finding of 45,X / 46,X,i(Xq). USG of Abdomen finding was absent uterus and ovaries[10]. Morgan T. found 2 cases out of 30 cases with loss of weight and short stature, their karyotyping findings showed 45,X and laproscopic findings showedbicornuate uterus and infantile uterus[11].
- Secondary Sex characters:
  In this study the development of secondary sexual characters (Table VI) was studied in all cases in which development of breasts, presence of axillary hairs and development of external genitalia were assessed. Out of 25 patients, 22 (88%) cases had normally developed, 2 (8%) cases had not developed, 1 (4%) case had poorly developed secondary sexual characters. In most of patients with absent uterus and cervix, other secondary sexual characters were found to be normal. This shows that presence or absence of uterus and cervix is not always associated with under development of secondary sexual characters. Chryssikopoulos et al, studied 77 cases of primary amenorrhea. Of the total number of patients, 31 had completely developed female phenotype, 22 had an insufficiently developed one and the remaining 24 patients were characterized by infantilism[12]. Oastler and Sutherland analysed 30 cases; out of them he found 11 cases with poorly developed and 6 cases with under developed secondary sexual characters[9].
  Dr.Purandare studied 109 cases; out of them 50 patients were with well-developed secondary sexual characters even in presence of rudimentary or absent uterus[4].
- Congenital anomalies:

In present study, out of 25 patients, 7(28%) had congenital defects.

Frequency of occurrence of congenital defects compared with other studies:

Author	Congenital defects
Mehrotra[13]	47.85 %
Roy[22]	28 %
Shah[15]	25 %
Present Study	28 %

Out of 25 cases, 7patients went for Diagnostic Laproscopy. 2 (8%) patients showed complete absence of uterus, 1(4%) patient had bicornuate uterus, 1 patient with imperforate hymen and 3(12%) patients had blind vaginal pouch. Other findings like associated renal malformations was present in 3(12%) cases and Steak ovaries in 1 case. Tullu MS et al, reported a case of, 18 year female who presented with primary amenorrhea, hypergonadotrophic hypogonadism and streak ovaries with hypoplastic uterus[16]. Jacobs P et al, reported a case of, 17 year old patients who presented with karyotyping finding of 46,X,i(Xq) and in laproscopy findings there was bicornuate uterus and infantile uterus[17]. Prognosis and management of primary amenorrhoea patients can be aided by diagnostic laparoscopy findings. Thus diagnostic laparoscopy is a useful investigation for diagnosing genital aplasia, hypoplasia and associated urinary tract anomalies.

- Cytogenetic study:
  Table(VIII)shows that in present study, 2 out of 25 cases (8 %) showed cytogenetic abnormalities. Turner's syndrome with 45,X was seen in 1 case and in another case primary amenorrhea due to structural abnormalities with 46,X,i(X)(q10) was detected. In remaining 23 cases, normal karyotype with46 XX was found.

Comparative analysis of cytogenetic findings in different studies.

Author	Normal karyotype	Abnormal karyotype	Detail of karyotype findings		
			45,XO	46,XY	Structural finding
Krumin AR,et.al[18];in March 1981.	51.6%	48.3%	10.9%	18.7%	Mosaicism –6.2%Del, iso-chromosomeetc. - 12.5%
Joseph A et al[19]; in 1984.	85.3%	29.57%	14.29%	-	Numerical or structural -10.2% &Chromosomal polymorphism - 4.67%
Mondal SK et.al[20]. in 2002	66.67%	33.33%	25%	8.33%	-
Wong MS et.al[10]. in 2005	75.5%	24.4%	16%	8.4%	-
In present study	92%	8%	4%	-	4%, 46,X,i(X)(q10)

Krumin'AR and co-worker, studied 64 patients with diagnosis of primary amenorrhea. Chromosome abnormalities or karyotype-phenotype non-conformity were found in 31 of 64 patients (48.4%). The following types of chromosomal pathology were found out: 7 cases with 45,X constitution; 3 cases with 45,X/46,XX; 1 case with 45,X/46,XY; 1 case with 45,X/46. XYq; 1 case with 46,XXq; 1 case of 45,X/46,XXq; 2 cases with 46,X, i (qX); 2 cases with 45,x/46,X, i (qX); 1 case with 45,X/46,XX, +mar. In 12 cases phenotypic women had a 46, XY karyotype[14]. Joseph A et. al, studied 150 cases with complain of sterility or primary amenorrhea. 63 patients had primary amenorrhea; 22 cases (14.7%) involved in this study had chromosomal abnormalities and 7 cases (4.7%) showed chromosomal polymorphism. Of the 107 females (44 sterile and 63 with primary amenorrhea), 11 (10.2%) showed numerical or structural sex chromosomal abnormalities. 5 patients (4.67%) showed chromosomal polymorphism (involving the paracentromeric and centromeric regions of chromosomes 1 and 9, double satellites and giant satellites)[19]. Mondal SK et. al, had studied 72 cases of primary amenorrhea to find out the proportion of genetic causes and to analyse different chromosomal pattern. They had found mullerian duct abnormalities in 27 cases (37.5%), gonadal agenesis in 13 cases (18.05%), turner stigmata in 18 cases (25%) and Y cell line in 6 cases (8.33%). Chromosomal aberration was seen in 24 cases (33.33%) and it was the second most common cause of primary amenorrhea after mullerian duct abnormalities[20]. Wong and co-workers had done cytogenetic analysis in 549 patients with either primary (n=237) or secondary (n=312) amenorrhea .Sex chromosome anomaly was found in 24.5% and 9.9%, respectively, of women with primary and secondary amenorrhea. In those with primary amenorrhea, male karyotype was identified in 8.4% and X-chromosome abnormalities in 16.0%[10]. Justus B and co-worker studied 39 patients with primary amenorrhea, with 12 of them exhibiting chromosomal aberrations. The classical chromosomal pattern of Turner's syndrome with 45, XO was exhibited in 7 patients, while mosaicism was displayed in the karyotype of 3. A typical iso-X-chromosome was established from a young girl without Turner's stigmata[21]. Tullu MS and co-worker, studied karyotype analysis of 100 metaphases using standard 'G'-banding technique revealed a pattern of 46, XX, t (1; 11) (q31; q25) suggestive of a balanced autosomal translocation involving the long arms of chromosomes 1 and 11[16].Genes essential for normal ovarian development are located on both arms of the X-chromosome. An abnormality in the number or structure of the X-chromosome results in a disturbance in the normal process of translation of genetic sex and the final determination of phenotypic sex. According to study Sanchita Roy (June-2015) the isochromosome i(Xq) form of TS was generally milder than classic Turner syndrome. A female with short stature, but without typical clinical findings of TS, should be evaluated for this chromosomal form, because 45, X karyotype can be diagnosed at birth due to typical dysmorphic features or cardiac abnormalities, but in isochromosome-X diagnosis may be delayed until childhood, adolescence or unfortunately until adulthood while evaluation done for short stature ,pubertal delay, primary amenorrhea and infertility. Early diagnosis is an important aspect of ideal treatment for these variant type of Turner syndrome[9]. Absence of menstruation in a teen-ager is an extremely stressful problem and should be handled with care and sensitivity. Counselling and psychological support are paramount in the management of these cases. Family history plays a major role in identifying autosomal inheritance. Careful pedigree analysis will give a clue to the underlying hereto-familial disorder in the affected individual as well as their family members. It is imperative to make a cytogenetic diagnosis not only to know the etiology but also for better management of the disease.

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