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PHENOTYPE- KARYOTYPE CORRELATION OF PATIENTS WITH PRIMARY AMENNORRHOEA IN NORTH KERALA

KEYWORDS

Primary amenorrhea, Structural anomalies of X chromosome, Noonan Syndrome, 17-alpha hydroxylase deficiency

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ABSTRACT

Objectives

The present study was carried out to establish the correlation between phenotype and karyotype of patients with primary amenorrhea of North Kerala.

Study settings and design

A cross sectional study of 190 phenotypic females within the age group of 14 to 35 years with a history of primary amenorrhea were included in the study. The study was conducted over a period of three years

Methods :

Peripheral blood lymphocyte cultures were set for each patient according to standard protocol and chromosomal analysis was carried out with the aid of CytovisionSoftware Version 7.

Results:

The results of the present study show that the incidence of chromosomal abnormalities in primary amenorrhoea cases of North Kerala is 21.1% .78.9% patients had 46XX karyotype. We also report one unique chromosomal anomaly- 46,X,t(X;4)(q22.1;p15.3)

Conclusions:

The results of the present study indicate that in all phenotypic females with primary amenorrhea, after excluding non genetic causes, karyotyping should be done for the precise diagnosis, management, prevention of complications in the future and counseling.

INTRODUCTION

Primary amenorrhea (**PA**) is the absence of menstruation in a woman of reproductive age. It is also defined as an absence of secondary sexual characteristics in a female who has attained the age of 14 but has not attained menarche or has secondary sexual characteristics but has not attained menarche by 16 years of age[1]

Amenorrhea is a symptom which may be caused by the congenital absence of the uterus and ovary, chromosomal abnormalities , hypothalamic or pituitary problems , extreme physical or psychological stress or a combination of these factors. According to World Health Organization, amenorrhea stands as 6thlargest major cause of female infertility. It affects 2-5 % of all women in the child bearing age[2].

Chromosomal abnormalities vary from 15.9% to 63.3% in patients with primary amenorrhea[3,4] Several cytogenetic studies have been conducted to establish the frequency and type of chromosomal abnormalities in primary amenorrhea. However no study has been carried out in the primary amenorrhea cases of North Kerala.

Methods

1 Sample collection:

The study subjects include patients with PA referred for chromosomal analysis to the Cytogenetic Laboratory attached to the Department of Anatomy of Government Medical College, Kozhikode, Kerala, India. The patients were referred from the departments of Gynaecology, General Medicine, Endocrinology, Urology and Plastic surgery. The study was carried out as per the norms of Institutional Ethics Committee . The age group of the subjects ranged from 14-35 years. Clinical information and results of relevant investigations were obtained from the respective departments.

2 Setting up of lymphocyte culture

About 1 to 2 ml of heparinized peripheral venous blood was collected from each patient aseptically. Each sample was labeled and a unique laboratory number was given

The blood samples were aseptically transferred into sterile culture tubes with 5 ml RPMI 1640 complete medium (Gibco) . Parallel cultures were set for each sample. The culture tubes were incubated in a CO2 incubator for approximately 72 hours. 73.5µl Colcemid (Gibco) was added to each culture tube at the 69th hour to arrest cell division at metaphase. After 20 minutes of incubation, the cell suspension was centrifuged for 8 minutes at 1000 rpm. The supernatant was discarded and the pellet was treated with hypotonic solution (0.075m KCl)drop by drop , mixing thoroughly with the help of Cyclomixer. The centrifuge tubes were incubated again at 37^{0}C for 15 minutes. The tubes were again centrifuged at 1000 rpm for 8 minutes. The supernatant was removed and 9 ml of freshly prepared prechilled Carnoy's fixative was added to the pellet while mixing on Cyclomixer. The tubes were kept in freezer for 10 minutes and then washed with freshly prepared Carnoy's fixative until the suspension becomes clear.

3 .Slide preparation

The slides were prepared by air drop method and GTG banding was done. The slides were stained with 1% Geimsa stain. Karyotyping was done with the help of Cytovision Software Version 7.Atleast 10-25 metaphases were examined for each patient to detect chromosomal anomaly and mosaicism.

Observation and Results

A total of 190 phenotypic females with PA were evaluated for chromosomal abnormalities. 150 (78.9%) patients had a normal

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karyotype (46XX) while 40 patients (21.1%)had chromosomal anomalies which included numerical as well as structural abnormalities. The details of karyotypes of the females and frequencies of different chromosomal anomalies are shown in **Table 1**

TABLE 1:Karyotype of patients with primary amenorrhoea

Sl. No	Karyotype	Details	No. of cases	Percentage	
1	**46 XX**	Mullerian agenesis	15	150	78.9
		RKMH syndrome	3		
		Congenitaladrenal hyperplasia	1		
		Noonan syndrome	1		
		Imperforate hymen	1		
2	**45 X**	Turner syndrome	18	9.47	
3	**46 XY**	Androgen insensitivity	5	6	3.15
		Congenital adrenal hyperplasia,(17 -OH deficiency)	1		
4	**Ring chromosome**	Mos46,X,r(X)[10]/45,X[50]	1	0.52	
5	**Inversion**	46,XX,inv(9)(p12;q13) 46,XX,inv(9)(p13;q13)	2	1.05	
6	**Isochromosome**	46,X,i(X)(q10)	5	6	3.15
		46,X,i(X)(p10)	1		
7	**Deletion**	46,X,del(X)(p11.22) 46,X,del(X)(p11.2)	2	1.05	
8	**Mosaics**	Mos 45,X/46,XY	2	4	2.10
		Mos 45,X/46,XX	1		
		Mos 46,X,r(X)/45,X	1		
9	**Translocation**	46,X,t(X;4)(q22.1;p15.3)	1	0.52	

In patients with normal karyotype, there were congenital anomalies like Mullerian agenesis, Mullerian hypoplasia, ovarian agenesis, congenital adrenal hyperplasisa with 17 hydroxylase deficiency, RMKH syndrome and Noonan syndrome. There was a case of imperforate hymen also.

In 40 cases (21.1%), there were chromosomal abnormalities. In 18 cases (9.47%), the karyotype was 45X. Six cases (3.15%) showed 46XY karyotype. Inversion was detected in 2 cases(1.05%), isochromosome in 6 cases(3.15%), deletion in 2 cases(1.05%) and translocation in one case(0.52%). Mosaicism was detected in 4 cases (2.1%). Ring chromosome was seen in one patient (0.52%)

DISCUSSION

Genetic disorders can have a health and economic impact on patients as well as their families, which indirectly affects the society also. Results of previous studies indicate that genetic causes of amenorrhoea accounts for approximately 45% of cases which may be a result of gonadal dysgenesis or mullerian agenesis.[5]

Several studies have been conducted to determine the frequency of sex chromosome abnormalities among patients with primary amenorrhoea[6,7,8]and it is estimated that chromosomal abnormalities are present in 46%-62% of patients with PA. These anomalies include X aneuploidy, male karyotype or structural anomalies of X chromosome[9]. The structural anomalies include isochromosome, rings, inversions, deletions andtranslocations.[10] Incidence of abnormal karyotype reported by various authors is highly variable; ranging from 15.9% to 63.3%[11], with the majority falling between 24%-46%[12]. The wide variation in the results may be due to the difference in sample size and selection of patients.

In the present study, the incidence of chromosomal anomalies is 21.1%. This is in accordance with Mohajertehran[10]et al (24.44%), Wong and Lam[3] (24.5%), Vijayalakshmi[6] et al (27.8%), Kalavathi[11] et al (25.8%), Safaei[12] et al (20%) and lower than Ramirez[13]et al (36.7%), Kong[14]et al (58.8%) and Butnariu[15] et al (54.56%).

A comparison of percentage of chromosomal anomalies detected by various authors is shown in **Table 2**

Table 2. Percentage of chromosomal anomalies in PA in different studies

Sl. No	Author (s)	Percentage of Chromosomal anomalies
1	Farnaz Mahajertehran et al.	24.44
2	Wong and Lam	24.5
3	Vijayalakshmi et al.	27.8
4	Kalavathi et al.	25.82
5	Ramirez et al.	36.7
6	Safaei et al.	20
7	Kong et al.	58.8
8	Butnariu et al.	54.56
9	Present study	21.1

For convenience of description, patients with primary amenorrhea who came to Government Medical College, Kozhikode, are grouped under six categories, based on their karyotypes.

1. 46XX
2. 45X
3. 46XY
4. Mosaics
5. Structural anomalies
   a) Inversion
   b) Isochromosome
   c) Deletion
   d) Translocation
   e) Ring chromosome

Females with 46 XX karyotype

Out of 190 patients with primary amenorrhea, 150 females (78.9%) had 46XX karyotype. Detailed investigations and physical examination carried out in the Departments of Gynaecology, General Medicine , Endocrinology and Urology revealed that, they had the following problems even though the karyotypes were normal.

1. Mullerian agenesis or hypoplasia - 15
2. RKMH syndrome - 3
3. Congenital adrenal hyperplasia - 1
4. Noonan syndrome - 1.
5. Imperforate hymen -1

1. Mullerian agenesis and Mullerian hypoplasia

Of the 190 cases studied, uterus was absent in **5** patients and hypoplastic in 10 cases. These patients had normal height and secondary sexual characters were developed. USG showed the presence of unilateral or bilateral ovaries. FSH and LH levels were normal

Developmental basis of anomalies of Uterus and vagina[16]:

The uterus and the fallopian tubes develop from the paramesonephric ducts present in the intermediate mesoderm. The ducts of the two sides fuse together to form the uterovaginal canal or uterine canal. The paramesonephric ducts give origin to the uterine tubes, the uterus and part of the vagina.

Anomalies of the Uterus:

1. The uterus may be completely or partially duplicated.
2. The lumen may be partially or completely subdivided by a septum
3. The entire uterus may be absent.

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4. One half of the uterus may be absent
5. The uterus may remain rudimentary
6. There may be atresia of the lumen either in the body or in the cervix
7. The cervix may be absent
8. Associated renal, skeletal or vertebral anomalies may be present.

Development of Vagina
During development of uterus and vagina, the lower end of utero-vaginal canal comes in contact with the urogenital sinus. These two are soon separated from each other by the formation of a solid plate of cells known as the vaginal plate.

The vaginal plate is formed as follows – Endodermal cells of the urogenital sinus proliferate to form two swellings called the sinovaginal bulbs. These bulbs soon fuse to form one mass. Most of the vaginal plate is formed from these sinovaginal bulbs .

The part of the vaginal plate near the future cervix is derived from mesodermal cells of the uterovaginal canal. The vagina is formed by the development of a lumen within the vaginal plate. The hymen is situated at the junction of the lower end of the vaginal plate with the urogenital sinus.

Anomalies of the vagina:
1. Vagina may be duplicated
2. The lumen may be subdivided longitudinally or transversely by a septum.
3. The vagina may be absent. This condition may or may not be associated with absence of uterus.
4. Vagina may have abnormal communication with the rectum (recto-vaginal fistula) or with the urinary bladder (vesico-vaginal) fistula.

RKMH Syndrome (Rokitansky -Kuster- Meyer-Hauser syndrome)
There were three cases of RKMH Syndrome. One patient, a 15 year old female, approached the Urologist with periodic hematuria. She was diagnosed to have utero vesical fistula.

A woman with a Rokitansky-Kuster- Meyer-Hauser syndrome lacks either the uterus, vagina or both. Renal and skeletal abnormalities can also occur. Secondary sex characters are normal since the ovaries are functional. RKMH syndrome is the etiology of primary amenorrhoea in 15% cases[17]. In the present study, 1.5 % females presented with RKMH syndrome, with normal 46XX Karyotype, well developed secondary sexual characteristics, normal ovaries and normal hormonal levels Females with RKMH syndrome may require an artificial vagina later inlife for sex.

Noonan Syndrome [18,19]
There was one case of Noonan syndrome. Patient presented with irregular scanty menstruation, short stature and webbed neck. Karyotype was 46XX. Secondary sexual characters were developed. Scanty menstruation was found to be due to malnutrition and anemia.

Noonan syndrome is inherited as autosomal dominant trait. Patients are usually fertile. The females in this group are often confused with those who have Turner syndrome because of superficial similarities. They have webbed neck, short stature, pulmonary valvular disease and occasionally mental retardation. Their karyotypes are 46XX.

Noonan syndrome is a relatively common autosomal dominant congenital disorder, named after Jacqueline Noonan, a pediatric cardiologist. Both girls and boys are affected. It is referred to as the male version of Turner's syndrome. Approximately 1 in 1000 to 1 in 2500 children worldwide are born with Noonan syndrome. Range and severity of features can vary greatly in patients with Noonan syndrome. In 85% cases, cardiac defects are detected. Gastrointestinal, respiratory, genitourinary, lymphatic, hematologic, developmental anomalies can also occur.

Shaw, Kalidas[20] et al followed 112 individuals with Noonan syndrome for a mean of 12 years (mean age 25.3, range 12-71). None of the patients died during this period, but several required medical interventions.

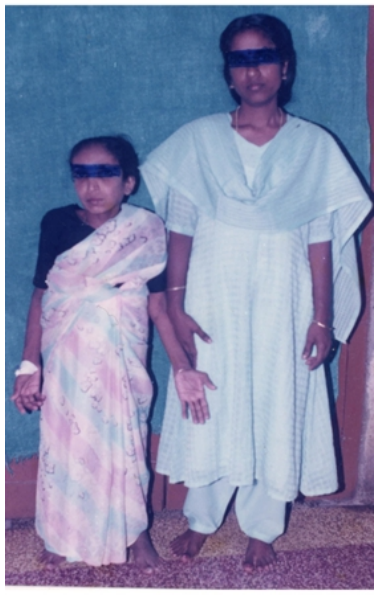
Imperforate hymen
This is a minor developmental anomaly which can be corrected surgically. In the present study, there was one case of imperforate hymen, with 46XX karyotype.

Turner Syndrome:
This was the most common chromosomal anomaly detected in the present study. A review of chromosomal abnormalities in 979 cases of amenorrhoeabyKalavathi[11],Chandra N[8] et al have found that the most common abnormal karyotype detected were 45X, 45X/46XX, 45X/46Xi(Xq) and 46XY. In the present study also, the most common chromosomal abnormality was 45X (9.47%), followed by 46 XY (3.68%), 45 X/46 XY and 45X/46XX (1.57%).

Abuhasan[21] et al (1999) in their study of 22 cases of Turner syndrome found 5 cases of 45X/46XX, 2 cases of 45X/46X,I (xq) and two cases of 45X/46XY karyotype.

18 cases out of 190 studied in Calicut Medical College were 45, X .There were two cases of 45X/46XYand one case of 45X/46XX; both patients were of short stature and were investigated for primary amenorrhoea.

The clinical features of Turner syndrome were different in each female. The commonest symptoms were short stature and primary amenorrhoea. Broad chest, widely spaced nipples, streak ovaries, webbed neck, short fourth metacarpals and metatarsals, coarctation of aorta, kyphoscoliosis **(Fig1)**, high arched palate, poorly developed secondary sexual characteristics were the other features. Turner mosaics showed a wide range of phenotypic features ranging from Turner features to that of normal females. Two patients with normal height were detected to have Turner mosaic karyotype during investigation for infertility.
Fig.1



Turmer Syndrome
A 40 year old female(45x) with a normal sibling.
patient has kyphoscoliosis,cubitus valgus,Right 4th short metacarpal,coarctation of aorta

One patient had prominent pterygium colli (webbed neck). She approached the Department of Plastic Surgery for getting the skin folds removed. The same patient had cubitus valgus, short stature, short fourth metatarsals and short fourth metacarpals **(Fig2a & b)**

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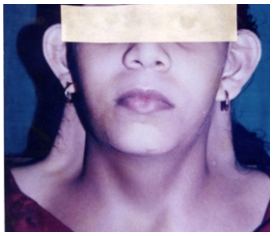

Fig.2a Patient with Turner syndrome showing Pterygium colli

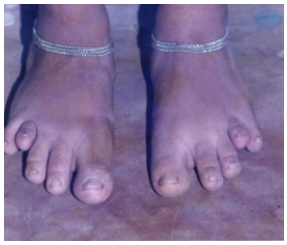

Fig.2b Feet of same patient showing short fourth metatarsals

Turner syndrome or Ullrich-Turner syndrome, should be included in the differential diagnosis of any phenotypic female with short stature and primary amenorrhoea. Early recognition of the clinical and chromosome features is important for optimal therapy.

Turner syndrome occurs in about 1 in 2500 female newborns. There is a high incidence of abortion (about 99.9%) in 45X embryos.

Clinical Features:

One of the cardinal features of Turner syndrome is the growth retardation. The subnormal height is a direct genetic effect of the chromosomal deficiency associated with the loss of all or part of the short arm of the X (Xp-).

Gonads, Hormones and Secondary Sexual Characteristics

In the absence of a Y chromosome, the gonad develops into an ovary. The ovaries contain primordial follicles during prenatal life and at birth. The 45X oocytes undergo attrition more rapidly than normal 46,XX oocytes. In most patients, by the age of puberty the ovaries are replaced by thin yellowish-white streaks that contain ovarian-like stroma but no follicles. Menarche occurs rarely and patients are usually sterile.A minority of the 45, X non-mosaic patients experience menarche, but usually end in early menopause.

Estrogen production is usually very low and gonadotropin excretion is high, underlining the primary nature of the ovarian failure.. Mental retardation is not a characteristic of the syndrome. Mile stones of development may be delayed. Arterial hypertension of unknown etiology, obesity, telangiectasis in the bowel, dissecting aortic aneurysms, non-insulin dependent diabetes mellitus can also be seen .

Karyotype abnormalities

The karyotype 45X is responsible for 40% to 60% of patients with Turner syndrome. Other karyotypes often associated with Turner syndrome involve structural aberrations of the X and Y chromosomes and mosaicism.

Management of Turner syndrome:

1. Early diagnosis
2. Detection of deafness, osteoporosis, telangiectasia, thyroid disease, diabetes, obesity, hypertension, aortic aneurysms.
3. Cyclic therapy with estrogen and progesterone should be initiated for the development of secondary sex characteristics and menses and the prevention of osteoporosis.
4. Recombinant human growth hormone should be given starting at 4 to 6 years of age and continued until the epiphysis has closed.
5. There is no known treatment to enhance the chances of ovulation or fertility. However, assisted reproductive techniques, chiefly in vitro fertilization with a donated ovum and then implantation into the properly hormonally prepared uterus has already been successful.

XY Females

Second commonest chromosomal anomaly detected in phenotypic females with primary amenorrhea was 46 XY karyotype. In the present study,6 cases out of 190 (3.15%) were detected to have 46XY karyotype.

Male karyotype in cases of phenotypic females with primary amenorrhoea range from 5.2%-30% in previous studies as shown in **Table 3**

Table 3:

Chromosomal abnormalities seen in cases of primary amenorrhea in different studies.

Our results are comparable to those of Akbar Safaei Mohd. Vasei and HosseinAyatollahi[12] (5.5%), Roy[22] et al (3.3%), Van Niekerk[23] (5.2%) and less than Wong[3] et al (8.4%) and Ten[24] et al (13.7%). Our values also go in accordance with the results of Butnariu[15] et al (5.2%), Ramirez[13](7.85%) and Wong[3] et al (8.4%) but much less than Mahajertehran[10] (18.2%), Kong[14](30%), Vijayalakshmi[6] (17.9%), Kalavathi[11](23.63%), Safaei[12](27.27%) and Ten24(13.7%).

Out of six cases , five had androgen insensitivity. Gonads (testes)were found in the inguinal canal in one patient. They were removed and the histopathology reports confirmed testicular tissue. But there

Karyotype results	Present study	Mohajer tehran et al	Wong et al	Kong et al	Vijaya lakshmi et al	Kala-vathi et al	Ram-rez et al	Safa ei et al	Butn arie et al	Ten et al
Frequency of abnormal Karyotype (number of cases)	21.1% (40)	24.44% (44)	24.5% (58)	58.8% (10)	27.8% (39)	25.82% (220)	36.7% (96)	20% (44)	54.56% (269)	30.8% (36)
X chromosome (homogeneous mosaics) aneuploidies	9.47% (18)	63.6% (28)	50% (29)	20% (2)	45.45% (100)	45.45% (100)	89.58% (92)	52.27%	82 .15% (221)	7.7% (9)
X chromosome unbalanced structural abnormalities	4.48% (8)	9.1% (4)	12.06% (7)	50% (5)	27.27% (60)	27.27% (60)	4.16% (4)	15.90%(7)	8.17%(22)	-
Marker chromosome	-	-	1.72% (1)	--	-	-	-	-	-	1.7% (2)
Mosaics X/XY and variants	2.62% (4)	9.1% (4)	1.72% (1)	-	-	3.63% (8)	-	4.54% (2)	3.34% (9)	-
46,XY	3.15% (6)	18.2% (8)	8.4% (20)	30% (3)	17.9% (7)	23.63% (52)	7.85% (3)	27.27% (12)	5.20% (14)	13.7% (16)
Other anomalies	1.04% (2)	-	-	-	-	7.23%	-	-	1.11% (3)	-

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were no malignant changes.

The sixth patient had a very rare anomaly, 17 alpha hydroxylase deficiency, which is discussed in detail below.

Case history:
21- year old phenotypic female admitted with primary amenorrhoea, headache and weakness of one half of the body, on examination showed the following features- Height – 167cm, masculine features, hypertension, hemiparesis (right), rudimentary nipples ,no axillary or pubic hairs , female external genitalia with a blunt vagina USG - No ovaries, no uterus , no adrenal or renal mass, no testes Other investigations revealed hypokalemia, marked reduction in urinary excretion of 17-Ketosteroid, 5µmol/24 hrs. (Normal value is 9-15µmol/24hrs). CT scan showed signs of hemorrhage in the basal nuclei Family history: Patient's sibling (18 years old female) also had hypertension, primary amenorrhoea, hypokalemia and decreased excretion of 17 ketosteroids.

Karyotype: This 21-year old phenotypic female had 46XY karyotype; and the 18 year old sibling, had 46 XX karyotype.

These siblings were diagnosed to have congenital adrenal hyperplasia and 17 α hydroxylase deficiency.17 Alpha-Hydroxylase deficiency is an uncommon form of congenital adrenal hyperplasia resulting from a defect in the gene CYP 17A1, which encodes for the enzyme 17 hydroxylase. This form of CAH accounts for less than 5% of the cases of congenital adrenal hyperplasia and is inherited as autosomal recessive manner with a reported incidence of 1 in 1,000,000 births .It leads to decreased synthesis of both cortisol and sex steroids with resulting increase in mineralocorticoid production. Symptoms include mild hypocortisolism, ambiguous genitalia in genetic males, failure of ovaries to function at puberty in genetic females and hypokalemic hypertension25

Pathophysiology:
17 hydroxylase converts pregnenolone and progesterone to their 17 hydroxyl formsas shown in the chart. The red arrows indicate the site of action of this enzyme.

Cholesterol
↓
Pregnenolone → Progesterone → Mineralocorticoids
↓ ↓
17 OH-Preg → 17 OH-Prog → → Glucocorticoids
↓
DHEA → Androstenedione → → Androgens, Oestrogens

Three reactions are common to both pathways (cholesterol side chain – cleavage; 3**β**-hydroxysteroid dehydrogenase / isomerase, and 17 **α**- hydroxylase). Impairment of any of these reactions results in deficiency of glucocorticoid and androgen synthesis.This in turn results in congenital adrenal hyperplasia (due to enhanced ACTH levels) and defective virilization of the male embryo (male pseudohermaphroditism).

17**α**- hydroxylase, 17,20 –lyase (p450 17 **α**) deficiency impairs the introduction of the 17-hydroxyl and the scission of the C 17,20 carbon bond that convert pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and androstenedione, respectively, which are mediated by a single enzyme, P450 17α encoded on chromosome 10.

Formation of both corticosterone and DOC (deoxycorticosterone- a potent salt retaining hormone) by the adrenal is elevated, and urinary 17 ketosteroids are low. Aldosterone secretion is low due to high plasma DOC and depressed angiotensin levels and returns to normal after suppressive doses of glucocorticoids.

Phenotypic women with 17**α** hydroxylase deficiency are characterized by 46 XX karyotype, hypogonadism, absence of secondary sex characteristics, hypokalemic alkalosis, hypertension and virtually undetectable hydrocortisone secretion, They have amenorrhoea, and the phenotype is that of a normal prepubertal woman.

In males, the deficiency results in defective virilization that varies from complete male pseudo hermaphroditism to ambiguous genitalia with perineoscrotal hypospadias and in some, gynecomastia. Adrenal insufficiency does not develop, since the secretion of both corticosterone (a weak glucocorticoid) and DOC (a mineralocorticoid) is elevated. Hypertension and hypokalemia are prominent (even in the neonatal period) and remit after suppression of the DOC secretion by glucocorticoid replacement.

A variety of point mutations, deletions and insertions in the P 450 17 gene on chromosome 10 have been characterized in affected individuals.

Treatment
Therapy with glucocorticoids and in some instances mineralocorticoids is indicated. The management of genital abnormalities depends on the individual case. In genetic females, there is no problem (except in diagnosis) in that affected individuals are raised as females and estrogen therapy is begun at the time of expected puberty to promote development of female secondary sex characteristics.

Whether newborn male with ambiguous genitalia should be raised as males or females depends on the anatomic defect. In general, the more severely affected should be raised as females and corrective surgery of the genitalia and removal of the testes should be undertaken as early as possible.

Structural Abnormalities of chromosomes:
In the present study five types of structural anomalies were detected, as shown in Table.1. There were 2 cases of inversion, 6 cases of isochromosomes, 2 cases of deletions,one case of ring chromosome(Mosaic Turner) and one case of translocation.

X:Autosome translocation(Fig 3)
We found a rare case of translocation, 46X,t(X;4)(q22.1;15.3), in a patient with primary amenorrhoea and short stature. This patient attained menarche at 15 years. After that, menstruation stopped, but she had withdrawal bleeding after administering hormones.

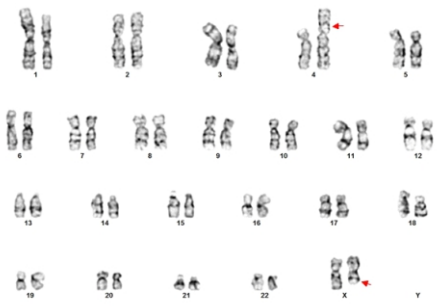

Fig.3 X : Autosome Translocation - 46, X,t(X;4)

X:Autosome translocations are rare; it is estimated to occur in 1/30,000 live births. Balanced reciprocal X:4 translocation in a female patient with early secondary amenorrhoea is reported by Jeffrey P.Phelan, Richard T.Upton, Robert L.Summitt[26]. They have reported 46X,rcp(X;4) (q 26; q21) type of translocation. In balanced X:autosome translocation, the normal X chromosome is inactivated preferentially to prevent deleterious monosomy of the translocated autosomal segment. Thus female carriers of balanced X:Autosome translocations are usually phenotypically normal Unbalanced X: Autosome translocation has been reported by Neeraj Gupta,

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Himanshu Goel and Shubha R.Phadke[27] They detected this chromosomal anomaly in a 5-month old female with multiple congenital anomalies and delayed milestones of development.

Isochromosomes

Various authors have reported isochromosomes. Our values (3.15%) are in accordance with results obtained by Akbar Safaei (1.8%) and more than that of Wong et al (0.4%) as shown in **Table3**

CONCLUSION

After the exclusion of non-genetic causes of primary amenorrhoea by Gynecologists and Physicians, the patients should be referred for genetic study.

Cytogenetic studies have shown that significant number of patients have sex chromosomal abnormalities. Genetic counseling should be given to these patients and their families explaining the possibilities of infertility and early menopause. Hormone replacement therapy can be started as early as possible so that risk of osteoporosis and coronary artery disease can be brought down. As the risk of gonadal malignancy in patients with XY gonadal dysgenesis is high, abdomen, pelvis and inguinal region should be scanned for the detection of site of gonad followed by its removal and histopathology examination.

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Figure Legends:

Fig.1 : A 40 year old female (45X) with her normal sibling. Patient has kyphoscoliosis ,Cubitus valgus and coarctation of aorta

Fig.2a: Patient with Turner syndrome showing Pterygium colli

Fig.2b: Feet of same patient showing short fourth metatarsals

Fig.3 : X : Autosome Translocation - 46,X,t(X;4)

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