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Original Research Paper

Medical Genetics

CLINICAL AND CYTOGENETICS ANALYSIS OF WOMEN THAT WERE SUSPECTED FOR TURNER SYNDROME IDENTIFIED AS 19.8% CHROMOSOMAL ABNORMALITY IN NORTH INDIAN POPULATION

Pooja Chauhan Ph.D., Research scholar, Centre for Genetic Disorders, Banaras Hindu University, Varanasi, India-221005.

Amit Kumar Rai* Ph.D., Assistant professor, Centre for Genetic Disorders, Banaras Hindu University, Varanasi, India-221005. *Corresponding Author

ABSTRACT

Purpose: The objective of present study was to know the contribution of different types chromosomal anomalies in manifestation of Turner syndrome. Turner syndrome is a chromosomal disorder mainly due to growth retardation and primary amenorrhoea. Cytogenetic analysis of cases referred for Turner syndrome is necessary for an early diagnosis which helps in genetic counselling to manage it in a better way. **Methods:** Total 237 cases suspected for Turner syndrome, were included in this study for duration of 7 years (2007-2014). We implemented the standard protocol for peripheral whole blood lymphocyte culture, chromosome preparation followed by G-banding. Chromosomes were analysed according to the guidelines of International System for Human Cytogenetic Nomenclature (2005). **Results:** After analysing 237 registered cases, chromosomal anomalies were seen only in 47 cases (19.8%). Careful clinical examination of patients with abnormal karyotype (n=47) revealed four major phenotypes i.e. growth retardation (n=19, 40.4%), primary amenorrhoea (n=19, 40.4%), primary amenorrhoea with growth retardation (n=6, 12.8%), and oligoamenorrhoea (n=3, 6.4%). Seven different types of chromosomal abnormalities were observed viz. Monosomy X (n=22, 46.8%), triple X syndrome (n=2, 4.2%), turner mosaic (n=3, 6.4%), ring chromosome (n=5, 10.6%), structural abnormalities with X chromosome (n=6, 12.8%), mosaic structural X abnormality (n=1, 2.1%), XY gonadal dysgenesis (n=8, 17%). **Conclusion:** This study revealed the frequency of most common clinical phenotype and different chromosomal abnormalities in patients suspected for turner syndrome. We observed growth retardation and primary amenorrhoea as most common clinical feature and monosomy of X chromosome as most frequent chromosomal abnormality in this cohort of study.

KEYWORDS : Turner syndrome, Growth retardation, Primary amenorrhoea, Gonadal dysgenesis, Cytogenetics.

INTRODUCTION:

Turner syndrome (TS), a variant of hypergonadotrophic hypogonadism, occurs due to partial or complete loss of X chromosome. World-wide frequency of TS is reported to be one in 2000 live female birth[1]. Primary clinical features associated with TS are short stature (Height less than 3rd or 4th percentile for that age)[2] and primary amenorrhoea (absence of menarche by the age of 14 years, with no development of secondary sexual characters). Manifestation of this syndrome is thought to be caused by a haploinsufficiency of the escape genes[3] in X-inactivation, as the X chromosome is enriched for genes expressed in the brain, skeletal muscle, and in sex and reproduction-related tissues[4]. Therefore, these women show phenotypes related to these organs. Renal malformation, cardiovascular defects, immunological problems, ophthalmological, otological defects and dermatological pathologies are also diagnosed in some of the cases.

Affected individuals have a variable range of functional and physical alterations likely due to a wide variety of chromosomal abnormalities, ranging from monosomy 45,X to various forms of mosaicism 46,XX/45,X with or without structural abnormalities of X chromosome. Monosomy X (45,X) represents half of the karyotype in this syndrome, and other half includes structural X chromosome abnormalities or mosaicism for a cell line containing a second sex chromosome[5].

A number of surveys from various parts of the world have been carried out to establish the contribution of sex chromosomal anomalies to the problem of TS. In this study, we have screened 237 cases, suspected for TS in order to determine the prevalence of different chromosomal abnormalities and to correlate it with various clinical outcomes of patients in population.

MATERIALS AND METHODS:

A total 237 cases were registered at our centre during 2007 to 2014, referred from University Hospital. The age of the cases recruited for the study, ranged from 8 to 36 years (Mean age-16.9±5.2). Pedigrees, clinical photographs and blood samples with written consent were collected from all the cases. Height was measured in inches by linear scale. Ultrasonographic reports of some of the cases were also collected from the Hospital. This work is approved by institutional ethical committee. Peripheral blood was collected from the patients and lymphocyte cultures were set up using RPMI-1640 culture medium pH 7.2 (Sigma-Aldrich, Inc., St. Louis, MI, USA) supplemented with 10% fetal bovine serum (Himedia), antibiotics, and phytohaemagglutinin-m (Sigma-Aldrich, Inc., St. Louis, MI, USA)) as per laboratory standard protocol. The cultures were incubated at 37°C in CO_2 incubator for 72 hours. Colchicine (Sigma-Aldrich, Inc., St. Louis, MI, USA) (0.02 □g/ml) was added in culture after 70 hours for arresting the cells at metaphase. After completion of 72 hours, cells were harvested after treatment with hypotonic solution. Cells were fixed in fixative (Methanol: Acetic acid 3:1 ratio) followed by G-banding by Saline–Trypsin–Giemsa (STG) method. Twenty metaphases with 450 G band resolution were observed under microscope and karyotyping was done with the help of automated karyotyping system using Ikaros karyotyping system—Metasystems software (Carl Zeiss Microscopy Gmbh, Göttingen, Germany). In case of mosaic pattern, we observed fifty metaphases. Chromosomes were analysed according to guidelines from International System for Human Cytogenetic Nomenclature[6].

RESULTS:

Chromosomal abnormalities were observed in 19.8% (n=47) cases rest showing normal karyotype (80.2%, n=190). Partial karyotypes were presented in this study (**Figure. 1,2**). Four different types of clinical phenotypes observed in chromosomally abnormal cases after medical examination were primary amenorrhoea (40.4%, n=19), growth retardation (40.4%, n=19), primary amenorrhoea along with growth retardation (12.8%, n=6) and oligoamenorrhoea (6.4%, n=3) (**Table I**).

Chromosomal abnormalities include both numerical (14.8%) and structural abnormalities (5.1%) (**Figure. 2, 3, 4**). Numerical abnormalities encircled X monosomy (45,X, 46.8%, n=22), X trisomy (47,XXX, 4.3%, n=2), sex reversal (46,XY,

17%, n=8), turner mosaic (46,XX/45,X, 4.3%, n=2) and mosaic (45,X/47,XXX, 2.1%, n=1) whereas structural abnormalities represented isochromosome (46,XiXq, 4.3%, n=2), mosaic structural X (46,Xdel(Xq)/45,X, 2.1%, n=1), deletion of short arm of X (46,Xdel(Xp), 2.1%, n=1), deletion of long arm of X (46,Xdel(Xq), 6.4%, n=3) and mosaic unbalanced structural X (46,Xr(X)/45,X, 10.6%, n=5) (**Table II**).

Table I: Clinical Correlations And Numbers In Tuner Syndrome (TS) Cases.

	N=	N=growth retardation≤ 141cm, Age≤13years (GR)	Primary amenorrheoa (PA)	GR+ PA	Other clinical referral #
Case screened for TS	237	81	119	18	19
Chromosomally normal case	190	62	100	12	16
Chromosomally abnormal case	47	19	19	6	3

(#Other clinical referral: edema, secondary amenorrhoea, premature ovarian failure, oligoamenorrhoea)

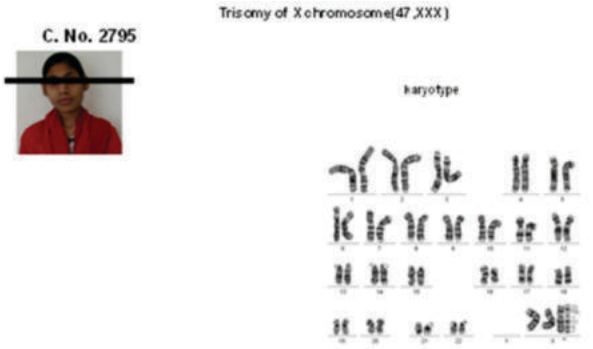

Figure.1 Clinical photo and karyotype in a patient of turner syndrome variants as trisomy 47,XXX.

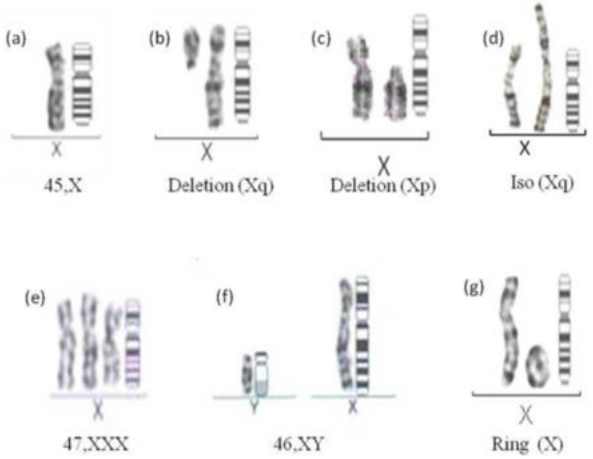

Figure. 2 Partial karyotypes in patients with turner syndrome: (a) Monosomy 45,X, (b) deletion of long arm of X chromosome del(Xq), (c) deletion of short arm of X chromosome del(Xp), (d) Isochromosome Iso(Xq), (e) trisomy 47,XXX, (f) male karyotype 46,XY, (g) ring X chromosome.

Table II: Cytogenetic Outcome And Its Analysis Of Turner Syndrome (n=47).

Chromosomal abnormalities	Karyotpye	Number of Cases	Frequency (%)
Numerical Abnormalities			
Monosomy of X	45,X	22	46.80%
Trisomy of X	47,XXX	2	4.25%
Sex reversal	46,XY	8	17.02%
Turner mosaic	46,XX/45,X	2	4.25%
Mosaic	45,X/47,XXX	1	2.12%
Structural abnormalities			
Isochromosome	46,X,iXq	2	4.25%
Mosaic structural X	46,Xdel(Xq)/ 45,X	1	2.12%
Deletion of short arm of X	46,Xdel(Xp)	1	2.12%
Deletion of long arm of X	46,Xdel(Xq)	3	6.38%
Mosaic unbalanced structural X	46,Xr(X)/45,X	5	10.63%
Total Cases		47	

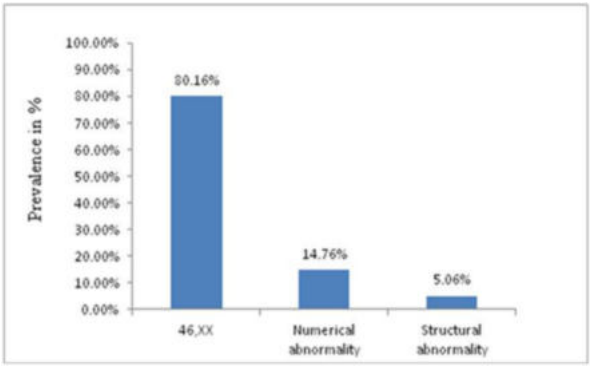

Figure. 3 Karyotype Frequency In Total Referred Cases.

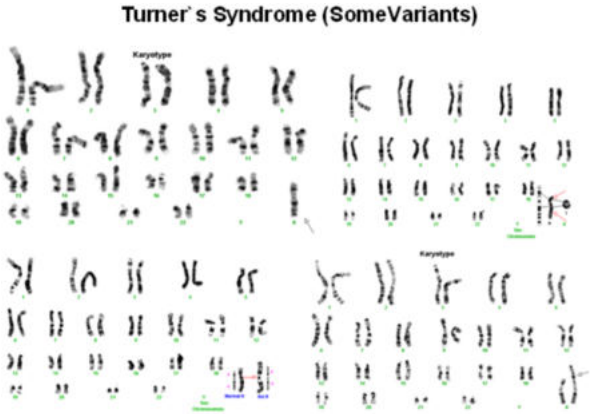

Fig. 4 Karyotypes Of Some Variants In TS Cases.

by previous studies in 46,Xdel(Xp) karyotype cases14. 8 cases of gonadal dysgenesis with karyotype 46,XY were also diagnosed which were referred due to complain of primary amenorrhoea. Two sisters of a family were diagnosed with primary amenorrhoea, swelling of

DISCUSSION:

The present study provides the frequency of chromosomal abnormalities in woman with TS in our population. The findings of this study are comparable with other previous studies reported from India. But as per our knowledge, this is the first report from India on large scale. The most common chromosomal abnormality observed in north Indian population, was 45,X (46.8%) which is similar to previous published data by Suri et al, [1995] but the second most frequent chromosomal abnormality observed was 46,XY (17%) in contrast to 46,XX/45,X mosaic as reported by previous study [7]. 31% cases were mosaic with one 45,X cell line and other cell line with second X or structurally abnormal X, compared with 30-50% are mosaic by Wiktor and Van Dyke[8]. We compared our frequency of chromosomal abnormalities with other previous reports in **Table III.**

The clinical phenotype of TS varies from case to case. Phenotype is not well anticipated by genotype, particularly in

the case of mosaicism[11]. This is particularly true for the various mosaicisms when the phenotype depends on the ratio of the different cell populations and their distribution in various tissues and organs[12]. The current study provided strength to the existing variability in genotype and phenotype manifestations. We also observed significant phenotype variation among patients with same abnormal karyotype. Patients with abnormal karyotype 45,X (n=22) showed three different types of manifestations; 8 patients with primary amenorrhoea, 10 with growth retardation while 4 with primary amenorrhoea along with growth retardation. Ring chromosome were also observed in 5 cases of which three cases presented growth retardation, one primary amenorrhoea along with growth retardation and one oligoamenorrhoea. One case with 46,Xdel(Xp) had growth retardation which was consistent with previous reported study[13]. Growth retardation might be due to haploinsufficiency of *SHOX* gene as reported thyroid gland and masculinised body. Both revealed showing same karyotype 46,XY. Ultrasonographic report (USG) report clearly showed absence of uterus and ovaries. Presence of testis and prostate with tumour were also seen in one sister. Gonadal tumours have been reported to present in 25% women with Y chromosome[15].

Table III: Publications Regarding Chromosomal Abnormalities In Turner's Syndrome.

	Total no. of cases	46,XX	46,XY	45,X	Mosaic 45,X	Partial X deletion or duplication	others
Suri et al., 1995	45	17.9 %	-	44.4 %	24.4%	13.3%	-
Malla et al., 2016	108	64.81 %	2.77 %	24.07 %	4.62%	2.76%	0.92%
Moka et al., 2013	146	54.1 %	8.9%	13.01 %	8.21%	4.77%	11.02%
Present Study	237	80.2 %	3.37 %	9.28 %	1.29%	4.64%	0.84%

We also registered 3 cases of Xq deletion of which two cases had growth retardation while one case manifested primary amenorrhoea with normal height. Studies on the deletion of the Xq have revealed that in most of these cases the chromosomal breakpoints occur within the critical Xq13q27 region[16]. Several scientists reported that patients with Xq deletion had normal stature because Xp arm maintains the stature in those patients. A study by Goldman *et al*., 1982 postulates the Xq deletion (deletion of Xq13-q26) generally results in ovarian failure[17].

Another two cases of triple X karyotype (47,XXX) were diagnosed with the problem of primary amenorrhoea. A study by Dewhurst et al presenting the similar case of triple X karyotype concluded a higher prevalence of ovarian failure in such cases[18]. Two cases of 46,Xi(Xq) were observed of which one case showed primary amenorrhoea whereas other growth retardation. One case 46,Xdel(Xq)/45,X was noticed with typical TS feature of edema, webbed neck, shield like chest, hypoplastic uterus, streaked ovaries and multiple neavi.

Majority of cases with growth retardation and primary amenorrhoea were diagnosed chromosomally normal. Cases with growth retardation might have another cause like deletion of *SHOX* gene or some alteration in function of this gene. Haploinsufficiency of SHOX gene is reported to be associated with short stature by Binder in 2011[14]. Most of the cases with primary amenorrhoea had ovarian dysgenesis with some other clinical features like absence of secondary sexual characters and blind vagina. Three cases also showed kidney related problems (One with ectopic kidneys, two with absence of left kidney). Inactivating mutations in follicular stimulating hormone receptor, luteinizing hormone receptor, thyroid stimulating hormone receptor, estrogen receptor and inhibin gene are also show association with ovarian failure[19,20]. These all genes are involved in hormone regulation through pituitary-hypothalamus-gonadal axis of ovarian cycle.

Here we present a study in a cohort of 237 cases, showing variety of karyotype and its frequency in TS patients. In our population, the most frequent chromosomal abnormality observed was 45,X followed by 46,XY. We also concluded that there is no obvious correlation between karyotype- phenotype manifestations. Variable phenotypes were noticed in patients with same karyotype and vice-versa.

The diagnosis inconsistency of TS is wide and can vary from endocrine disorders, genetic anomalies, psychological and structural abnormalities. Thus, karyotyping is one of the standard diagnosis procedures for determining chromosomal abnormalities involved in this disorders[21, 22, 23]. If chromosomal abnormalities are detected, a full explanation should be given to the patient by a geneticist or gynecologist with experience in genetics. Counselling should cover the risk of premature menopause for patients with TS and the use of hormonal replacement therapy, the possibility of infertility in the future children of patients with mosaic Turner, and the risk of gonadal tumour in patients with XY gonadal dysgenesis. A significant number of patients had sex chromosomal abnormalities, thus early cytogenetic analysis is advisable to guide further management. Early diagnosis will enable early involvement and early psychological counselling to the patient as well as the parents, which in turn will help to improve their quality of life.

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