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PHENOTYPIC DISTRIBUTION OF TURNER SYNDROME IN BANGLADESH: AN ANALYSIS OF 446 CASES

Immunology

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ABSTRACT

Background: Turner syndrome (TS) is the most common sex chromosomal numerical genetic abnormality that affects approximately 1 in 2,500 to 1 in 3,000 live female births corresponding to approximately 1.5 million women worldwide per year. There is a wide variation of clinical features seen in females with short stature, gonadal dysgenesis, neurocognitive disorder, lymphedema, and characteristic dysmorphic features. The aim of this study was to investigate cytogenetic and phenotypic profile of Turner syndrome in a group of referred patients with suspected genetic disorders.

Methods: This observational study was carried out at the Cytogenetic Laboratory of the Department of Immunology BIRDEM General Hospital for a period of seventeen years from 2000 to 2016. A total of 9,216 patients suspected for different chromosomal abnormality (e.g. numerical chromosomal disorders, primary amenorrhoea, ambiguous genitalia etc.) were included in this study referred by physicians of various discipline from different areas of Bangladesh. From the patients, referred for cytogenetic study, detailed family history and physical findings were noted. Complete genetic examination and pedigree construction was done to exclude non-chromosomal causes of anomaly. For cytogenetic analysis, peripheral lymphocyte culture by the standard method using the G-banding technique was employed.

Results: In these study 446 (4.83%) female patients of referred patients was diagnosed as TS in cytogenetic study and most of them (36.3%) were diagnosed in their adolescent period between 11-15 years of age group. Classical cytogenetic form of TS – 45, X (56.8%) were most common followed by other mosaics. To see association of age and different phenotypic feature (neckwebbing, carrying angle, short stature, developmental milestone) with various cytogenetic form of TS we did Chi square with Fisher's Exact Test and we found all variables had significant association. To find out the prediction ability of variables multiple linear regression was done and this model can predict the variation up to 11.4% as well as statistically significant ($p< 0.05$). Among the variables respondent's age was the strongest predictor ($p=0.000$, CI: 0.071, 0.211) followed by neck webbing ($p=0.002$, CI: 0.118, 0.518) and short stature ($p=0.010$, CI: 0.057, 0.414) while controlling the other variables.

Conclusion: TS diagnosis in early age before puberty is crucial from other related disorders and thereby helps in improving the quality of life by providing appropriate and timely treatment. Therefore, we need to focus to improve our diagnostic capacity for TS for proper intervention.

KEYWORDS

Turner Syndrome, Cytogenetic Analysis, Phenotype, Genotype.

Introduction:

Turner syndrome (TS) is the most common sex chromosomal numerical genetic abnormality that results of complete or partial monosomy of the X chromosome. [1] TS affects approximately 1 in 2,500 to 1 in 3,000 live female births corresponding to approximately 1.5 million women worldwide per year.[2] Approximately half of them have monosomy X (45,X), and 5 to 10 percent have a duplication (isochromosome) of the long arm of one X (46,X,i(Xq), some of them have deletions of short and long arm of X chromosome. Rest have mosaicism for 45,X, with one or more additional cell lineages.[3]

Clinicians suspects TS prenatally when they found pedal edema in fetus on ultrasonography. Triple screening of maternal serum also done to see abnormal levels of human chorionic gonadotropin, unconjugated estradiol, and alpha-fetoprotein. In a child who has 45, X or mosaicism for 45,X diagnosed after birth is usually identified because of phenotypic features suggestive of TS.[4]

There is a wide variation of clinical features seen in females with TS, ranging from the severe phenotype with short stature, gonadal dysgenesis, neurocognitive disorder, lymphedema, and characteristic dysmorphic features, to women with only a mild reduction in final height, or premature ovarian failure. Diagnosis of TS may be delayed until adulthood in up to 10%. In most cases patients with TS diagnosed either in adolescence when they fail to enter puberty or in adulthood with recurrent pregnancy loss.

The diagnosis is made on the basis of a chromosomal analysis. A peripheral lymphocyte karyotype is routinely analyzed and is diagnostic in the majority of cases. Detection of mosaicism depends on the proportion of cells present from the additional cell lineages. In routine karyotyping, more than 20 cell divisions were counted, since this number is sufficient to detect mosaicism. When diagnosis of TS suspected clinically but the result of routine testing is normal, then increase the number of cells counted to 100 and performing a skin biopsy for karyotyping of fibroblasts are indicated to find out mosaicism for an abnormal cell lineage.

Which part of chromosomal regions and genes responsible for the physical characteristics of TS remains uncertain.[5] The physical manifestations of TS has been hypothesized either due to the absence of two normal sex chromosomes before X-chromosome inactivation or to haploinsufficiency of genes in the pseudoautosomal regions of the X or Y chromosome, as well as to aneuploidy itself. [6,7] Both the short arm and the long arm of the X chromosome contain genes important for ovarian function but loss of interstitial or terminal long-arm material of the X chromosome (Xq) can result in short stature, typical skeletal change and primary or secondary ovarian failure. [8] Loss of Xq results in haploinsufficiency of the short stature–homeobox (SHOX) gene, located in the pseudoautosomal region of Y and Xp. Loss of a region at Xp22.3 appears to be associated with the neurocognitive problems in Turner's syndrome.[9] Loss of the testis-determining factor (SRY) gene locus on the short arm of the Y

chromosome (e.g., 46,X,del(Yp)) also leads to the phenotype of TS in male. A region on Xp11.4 has been proposed as critical for the development of lymphedema. [10]

Different types of mosaicisms showed correlation with phenotypic features. Women with mosaicism for 45,X/46,XX are marginally taller than other women with Turner's syndrome. Patients with a karyotype of 45,X/46,XX or 45,X/47,XXX are the most likely to have spontaneous menarche and fertility.[11] The presence of an isochromosome Xq suggests an increased risk for hypothyroidism and inflammatory bowel disease.[12] The presence of a ring chromosome associated with an increased risk of mental retardation and atypical phenotypic features.

The majority of women with TS require long-term estrogen replacement therapy. After the induction of puberty and the completion of growth, females with TS should be maintained on cyclical estrogen-progesteron therapy. Estrogen replacement therapy in adults with TS is important in the prevention of osteoporosis and in reducing risk factors for atherosclerosis.[13] In addition, it has been shown recently that estrogen replacement may improve aspects of cognitive function in women with TS.[14]

Women with TS are at risk for a number of medical problems that require care throughout adulthood. After the completion of puberty and growth treatment, women with TS should be followed up by a multidisciplinary team equipped to manage the specific medical problems associated with the syndrome. Long-term morbidity associated with endocrine dysfunction, so it is necessary for endocrinologists to become skilled in other aspects of the syndrome to provide a holistic approach to long-term care. Ideally, cardiologist, surgeon, audiologist, genetic councilor, psychologist, and fertility specialist should be made available in one clinic to provide one stop service. With increasing awareness of these issues and the development of more dedicated facilities, we can improve life expectancy and quality of life of patients.

Materials and Methods

This retrospective observational study was conducted in the Cytogenetic Unit of the Department of Immunology at BIRDEM General Hospital, Dhaka for a period of seventeen years from 2000 to 2016. A total of 9,216 patients were included in this study. These patients were referred from different area of Bangladesh for suspected chromosomal abnormality. 446 suspected patients of TS were referred by the clinicians of various disciplines, but we also diagnosed TS by karyotyping out of this referral. The suspected chromosomal abnormality includes patients with numerical chromosomal abnormality (e.g. Down's syndrome, Klinefelter's syndrome and Turner's syndrome), ambiguous genitalia, primary amenorrhea (e.g. X-chromosome deletion), adrenogenital syndrome. Among them the suspected TS patients were mainly referred with the complaints of short stature, gonadal dysgenesis, neurocognitive disorder, lymphedema, and characteristic dysmorphic features. All the patients were subjected to complete genetic examination and pedigree construction was done to exclude non-chromosomal causes of anomaly. Detailed history and physical findings were also noted.

The study followed the conventional peripheral lymphocyte culture by the standard method using the G-banding technique. The protocol employed for karyotyping was as follows: about 2 ml of heparinized blood was collected in a syringe from peripheral veins of the referral patients. Lymphocytes were grown in RPMI (Roswell Park Memorial Institute)-1640, media containing antibiotics (penicillin and streptomycin) and 15% serum supplementation (fetal bovine serum). The phytohaemagglutinin (PHA) was added as a mitotic stimulant and the samples were incubated for 72 hours at 37°C in 5% CO2 incubator (Forma Scientific, USA). The cells were arrested at metaphase stage of cell cycle with 0.1% colchicine after the incubation. Then after one hour of incubation (with colchicine) the cells were treated with KCL hypotonic solution. After that, the cells were fixed by three times wash with fixative solution (3:1; methanol: glacial acetic acid). All the reagents used were from Sigma Aldrich, Germany. The slides were then stained with Giemsa and air dried. Chromosome analysis was done under 100X magnification. At least 30 metaphase spreads were screened for each patient.

Results:

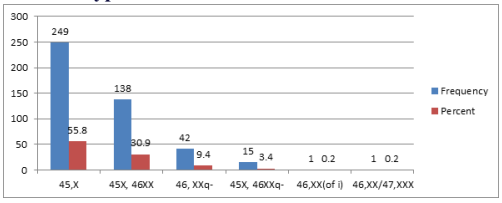
In this study total 9,216 suspected patients were analyzed for karyotyping and TS were detected in 446 (4.83%) female patients. The age limit of the patient ranges from 4 months to 40 years. Most of the patients (36.3%) were in the age group of 11-15 years (table – I)

Table I: Age distribution of patients detected TS in Karyotyping.

Age group	Frequency	Percentage (%)
Less than 1 yrs	24	5.4
1-5 yrs	16	3.6
6-10 yrs	34	7.6
11-15 yrs	162	36.3
16-20 yrs	148	33.2
More than 20 yrs	62	13.9

Among the patients diagnosed as TS in karyotyping showed different sex chromosome aneuploidy. Most common was the classical form of TS - 45, X (55.8%) followed by a mosaic form 45, X / 46, XX (30.9%), 45X, 46XXq- (3.4%) and 46XX, 47XXX (0.2%).. Rare variety, partial arm deletion and isochromosome such as 46 XXq- and 46XX (of i) was also found (figure- I).

Figure I: Genotype variation of TS



Disparity in developmental milestone with wide variety of clinical phenotype found in turners female.

Table 2: Percent distribution of different variables.

Clinical phenotype	Variation	Frequency	Percent (%)
Stature	Short	205	46.0
	Normal	241	54.0
Neck webbing	Present	143	32.1
	Absent	303	67.9
Obesity	Present	243	54.5
	Absent	203	45.5
Carrying angle	Normal	322	72.2
	Increased	124	27.8
Developmental milestone	Normal	245	54.9
	Delayed	201	45.1

To find out the prediction ability of variables (age, carrying angle, developmental milestone, neck webbing, stature) multiple linear regression was done. The test reveals that the model is statistically significant (p= 0.000) and this model can predict the variation up to 11.4%. Among the variables respondent's age was the strongest predictor (p=0.000, CI: 0.071, 0.211) followed by neck webbing (p=0.002, CI: 0.118, 0.518) and stature (p=0.010, CI: 0.057, 0.414) while controlling the other variables.

Table 4: Multivariate analysis of factors

Characteristics	Unstandardized Coefficients		Standardized Coefficients	t	Sig	n= (446) 95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Age	0.141	0.036	0.179	3.947	0.000	0.071	0.211
Carrying angle	-0.198	0.106	-0.092	-1.870	0.062	-0.406	0.010
Developmental milestone	0.040	0.087	0.021	0.457	0.648	-0.131	0.211
Neck webbing	0.318	0.102	0.154	3.123	0.002	0.118	0.518
Stature	0.235	0.091	0.122	2.591	0.010	0.057	0.414

Discussion:

In this study total 446 suspected patients of TS was referred by the clinicians of various disciplines and they were investigated for numerical chromosomal abnormality by Karyotyping. All were female patients categorized according to their age group and most of the patients (36.3%) were in the age group of 11-15 years. A Belgium study group of paediatric endocrinology showed that in 22% of the girls were diagnosed after the age of 12 years with the complaints of height deficit [15]. Our age groups of Turner's patients were similar with this study and at this age parents of the patients become concerned about short staure and delayed puberty of patients. In our study 46% of patients come with short stature and average height were below 4 ft 7 in (140 cm). In our country it is easy to misinterpret small size of these patients as due to constitutional delay, but when they face delayed puberty problem then they further investigated. Patients with Turner's mosaicism can reach normal average height, so we recommend that a cytogenetic analysis should be considered in all girls with unexplained short stature. Recombinant human growth hormone in patients with Turner's syndrome can be given after appropieate diagnosis.

In our study classical form of TS - 45, X was most common (55.8%) followed by a mosaic form 45, X / 46, XX (30.9%), 45X, 46XXq- (3.4%) and 46XX, 47XXX (0.2%). Classical form of TS presented with common clinical features such as short stature, neck webbing, obesity, carrying angle and delayed developmental milestones. But in case of mosaicism gonadal dysgenesis, neurocognitive disorder, lymphedema, characteristic dysmorphic features, cardiac diseases, renal malformation and autoimmune thyroid diseases presented along with classical features. Rare variety, partial arm deletion and isochromosome such as 46 XXq- and 46XX (of i) was also found in our study.

Although clinical features of TS have been well defined, the severity of phenotypic features in TS individuals differs according to the underlying chromosomal constitution [16]. We found significant association of TS with common clinical features (age, carrying angle, developmental milestone, neck webbing and short stature). Then we looked for prediction ability of variables by multiple linear regression analysis and this model can predict the variation up to 11.4%. Among the variables respondent's age was the strongest predictor (p=0.000, CI: 0.071, 0.211) followed by neck webbing (p=0.002, CI: 0.118, 0.518)& stature (p=0.010, CI: 0.057, 0.414) while controlling the other variables. The cause of obesity in females with TS is unknown, but may be related, in part, to estrogen deficiency. TS patients complaints with reduced physical fitness which may partially improved by sex hormone replacement. Sensorineural hearing loss and occurrence of auricular anomalies were significantly increased in 45,X patients.

Ovarian failure with subsequent estrogen deficiency is a common feature of TS. Although approximately 30% of girls with TS have evidence of some spontaneous pubertal changes, many of these girls will not progress fully through puberty and only 16% will have spontaneous menses with eventually secondary amenorrhea [17]. Menstrution are more likely to occur in girls who are mosaics rather than those with a classical 45X karyotype. Thus long-term estrogen replacement therapy is the main part in management of TS patients. The most serious, life-threatening consequences of X-chromosome haploinsufficiency involve the cardiovascular system. This is most apparent during fetal development, where major cardiac defects result in high mortality for fetuses with a 45,X karyotype.

Our study has some limitations. First, it was a retrospective observational record based study with data collection relied upon reviewing the medical records with the possibility of missing data and loss from follow up. Second, it was conducted in one center that might not represent other health care centers in Bangladesh.

Conclusion:

Patients with TS appear to have a decreased life expectancy due to complications of heart disease, kidney diseases and other hormonal problems. Chromosomal analysis for all suspected cases of TS should be done as early as possible for appropriate management early in life. It is important to find out phenotypic correlations with varieties of genotype in patients with Turner syndrome. The effect of different X chromosome abnormalities on the severity of clinical features should be studied carefully by using various modern techniques (FISH, Gene sequencing) on larger population.

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