RESEARCH PAPER

Karyotype-Phenotype Correlation in Turner Syndrome at a Single Center in Eastern India

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Objective: To describe clinical features in Indian girls with Turner syndrome along with the phenotype-karyotype correlation. **Methods**: 103 girls with Turner syndrome were divided into karyotype-groups: Classic (45X), 45,X/46,XX mosaics, isochromosomeXq (46,X,iXq and 45,X/46,X,iXq mosaics), 45,X/46,XYmosaics and structural defects, and analyzed for phenotypic differences. **Results:** Majority (44.1%) had classic karyotype followed by isochromosome-Xq (26.5%). Classic Turner syndrome had higher prevalence of most skeletal and cutaneous stigmata, cubitus valgus (68.3%) and multiple nevi (68.2%) being the commonest. Bicuspid aortic valve was most common in 45,X/46,XX mosaics (5/15, 33.3%), and aortic coarctation in classic TS (3/42, 7.2%). Congenital renal anomalies occurred mostly in classic TS (6/42,14.3%). Overt hypothyroidism, conductive deafness and recurrent otitis media were commonest in isochromosomes (P<0.03). 45,X/46,XY mosaics had highest IQ (P<0.005). **Conclusion:** We report some novel associations of karyotype with non-endocrine parameters in Turner syndrome. In resource-limited settings, underlying karyotype may help prioritize screening investigations in girls with Turner syndrome.

Keywords: *Congenital anomalies, Karyotype, Skeletal stigmata, Turner syndrome.*

Turner syndrome is characterized by short stature and multiple skeletal deformities, gonadal failure, congenital anomalies of cardiovascular and urinary system, neurocognitive abnormalities, autoimmune diseases, metabolic abnormalities and osteoporosis [1]. There is variability in the clinical manifestations of Turner syndrome depending on the karyotype and other factors like parental origin of the X chromosome and epigenetic modification [2]. The extra-endocrine manifestations in Turner syndrome like the cardiac or renal deformities and autoimmune disorders are important to detect early for timely intervention and improving longevity. Results of studies trying to correlate genotype with phenotype in Turner syndrome have often been inconsistent although there are some established associations like increased autoimmune disorders in isochromosomes, mental retardation in ring chromosomes and an overall milder presentation in 45,X/46,XX mosaics compared to classic Turner syndrome (45,X) [3-5].

There are few studies on Turner syndrome from India [6,7]. The current study reports the skeletal stigmata and different congenital anomalies, otologic and neurocognitive aspects of Turner syndrome in India and analyzes inter-karyotype phenotypic differences.

METHODS

Karyotype analysis was performed according to the International System for Human Cytogenetic Nomenclature (ISCN, 2005) guidelines [8] on 20-30 metaphase cells. Ethical clearance for this study was obtained from the institutional ethics committee of IPGME&R, Kolkata. Skeletal stigmata were assessed as per standard definitions [9]. Echocardiography with color doppler assessment was used to detect congenital cardiac malformations and assessment of aortic root diameter. For girls older them 15 years, Weschler adult intelligence Score (WAIS IV) was used whereas for those aged between 5-15 years, the Malin intelligence scale for Indian children (MISIC), a validated Indian adaptation of Weschler intelligence scale for children (WISC), was used for intelligence testing [10].

The patients were grouped into categories depending on their karyotype including classic-Turner syndrome (45,X), 45,X/46,XX mosaics, 45,X/46,XY mosaics, isochromosome-Xq (46,X,iXq or 45,X/46,X,iXq), structural defects of X (del-Xq or ring chromosomes) and complex karyotypes. The results obtained for the parameters were analyzed for differences between classic and non-classic Turner syndrome and among the first four karyotypes.

Statistical analyses: Statistical analyses were done using GraphPad Prism v.6e. Differences between karyotypes were assessed using unpaired t-test, ANOVA, or Chi-square test as applicable. *P* value <0.05 was considered as significant.

RESULTS

Of 103 patients with Turner syndrome, majority (44.1 %) were classic Turner syndrome followed by those with isochromosome-Xq (26.2%) and 45,X/46,XX-mosaics (17.6%). The mean (SD) age of presentation was 14.8 (3.97) years, upto 98% of the patients presenting due to gonadal failure (74%) or short stature (24%). Only 2% of the girls presented due to associated comorbidities or complications, chiefly cardiac. The youngest age of diagnosis was 6 years; diagnosed during evaluation for coarctation of aorta.

Cubitus valgus (68.3%) and multiple nevi (68.2%) were the most prevalent stigmata. Classis TS had a significantly higher prevalence of short fourth meta-carpals/metatarsals, high arched palate, edema of hands/feet, low posterior hairline, low set ears and shield chest. We also noted some atypical stigmata like absent terminal phalanges of digits and terminal transverse defect of lower limb.

Congenital cardiac malformations were found in 21.5% patients, most common malformation being bicuspid aortic valve (*n*=6) followed by septal defects (ASD/VSD) (*n*=6) and coarctation of aorta (CoA) (*n*=4). Others included root dilatations with regurgitation of aortic and tricuspid valve and mitral valve prolapse. Four patients had multiple cardiac malformations. 45,X/46,XX mosaics had higher prevalence of BAV (5, 33.3%), (*P*=0.004 vs classic Turner Syndrome). Most cases of aortic coarctation occurred in those with classic Turner syndrome (75%) (**Table I**). Classic Turner syndrome had the highest aortic root diameter (27.55 mm +/- 4.03). ECG abnormalities were seen in 11.2% - mostly left-ventricular-hypertrophy (LVH) or Right-axis-deviation (RAD) secondary to coarctation of aorta or septal defects, or non-specific ST-T wave changes. Hypertension was seen in seven patients, out of which three had coarctation of aorta. Neither hypertension nor ECG changes had any karyotype preponderance.

Classic Turner syndrome had a slight majority of congenital anomalies of kidney and urinary tract (CAKUT) (19 % vs 6.1 % vs non-classic TS, *P*=ns). Renal anomalies were mostly seen in classic TS (14.3% vs 2.4%, *P*=0.04). Horseshoe kidneys (9.5%) were commonest, followed by unilateral fused kidneys (4.8%). Duplicated pelvicalyceal system, PUJ abnormalities occurred equally in classic and non-classic karyotypes.

Upon pure tone audiometry testing, 11% had conductive hearing loss (HL) whereas sensorineural hearing loss (SNHL) was seen in 18.2% and mixed HL in 7.3%. Upto 18.4% had recurrent otitis media. There was a significantly higher prevalence of recurrent otitis media and conductive deafness in isochromosomes (both 47.3%, P_{both}<0.03). Classic TS had slightly higher prevalence of sensorineural hearing loss (SNHL) (27.3%, *P*> 0.05).

Celiac screening with serum tissue-transglutaminase IgA-antibody were negative in fifty asymptomatic patients tested (with normal total IgA levels). One patient with malabsorptive symptoms revealed villous atrophy and lymphocytic infiltrates on duodenal biopsy.

Thyroid antibodies (anti- TPO Ab and/or anti-Tg Ab) were found in 52.9% of the girls, 28.2% had overt hypothyroidism and 23.5% had subclinical hypothyroidism. One patient with classic TS had Graves' disease.

Table I Prevalence of Phenotypic Abnormalities in Different Karyotype of Turner Syndrome (*N*=103)

Karyotype group (n)	*Classic TS (n=43)*	*Non-classic TS (n=55)*	*XO/XX mosaics (n =18)*	*Iso-chromosome Xq (n=25)*	*XO/XY mosaics (n=6)*
Cardiac malformations, *n* (%)	23.8	20	46.7[a]	4.1	16.7
Aortic root diameter (mm)	23.8 (2.4)[a]	23.5 (2.5)	24.8 (3.1)	22.5 (1.8)	23.8 (2.4)[a]
Congenital anomalies of kidney and urinary tract, *n* (%)	19	6.1	6.7	0	0
Conductive hearing loss	10.8	31.6[a]	23	47.3[b]	0
Verbal IQ	88.2 (10.4)	87.7 (19)	71.1 (17)[c]	91.8 (13.8)	107.3 (11.6)[a]
Performance IQ	75.6 (8.4)	75.2 (14.9)	62.3 (12.2)[c]	78.9 (12.9)	89 (2.2)[a]
Arithmetic scores	79.9 (10.3)	77.9 (17.7)	62.9 (17.1)[c]	81.4 (12.5)	96.8 (8.1)[a]

All values in mean (SD) or as stated; TS: Turner syndrome; IQ: Intelligence quotient; [a]significant difference from classic TS (P<0.004); [b]significantly higher than classic TS (P=0.02); [c]significantly lower than classic TS (P<0.005).

WHAT THIS STUDY ADDS?

- This study provides data on clinical features of Turner syndrome from a large cohort of Indian patients.
- Karyotype may help prioritize some screening investigations in resource-constrained settings.

There were no significant differences in anti-thyroid antibody positivity or prevalence of autoimmune thyroid disease (AITD) [TPO/Tg positive and not euthyroid] among the karyotypes. Overt hypothyroidism was significantly higher for isochromosomes (52%, P=0.002) whereas subclinical hypothyroidism and euthyroidism with TPO/Tg-Ab positivity were both slightly higher in XO/XY mosaics (50% and 16.7%,P_{both}=ns).

Out of fifty patients studied, 42% had normal VIQ, only one patient had normal PIQ. 58% had a discordance between VIQ and PIQ (VIQ–PIQ>10). 45,X/46,XY mosaics had the highest and 45,X/46,XX mosaics the lowest Verbal and Performance IQ as well as arithmetic scores (P_{both}<0.005). We had a single case of ring chromosome in our cohort who had extremely low PIQ as well as extremely low VIQ. Of those with extremely low PIQ (PIQ<70), 28.6% were classic TS while 50% were 45,X/46,XX mosaic TS.

DISCUSSION

Though several studies have reported an increased severity of stigmata in classic TS, the differences of Turner's stigmata in different karyotypes have less clinical relevance. We had similar findings and in addition, we found some atypical skeletal stigmata like absent terminal phalanges of digits and terminal transverse defect of lower limb which might have a biologically plausible explanation related to compression of the developing limb bud by in-utero lymphedema or SHOX haploin-sufficiency.

CoA was seen almost exclusively in classic TS which is also reported in the Turkish registry [11] and cardiac malformations were overall more in classic TS in a study from Saudi Arabia [5]. There are predominantly two mechanisms leading to CCMs in TS. The first is jugular lymphatic-sac obstruction causing distension of the thoracic-duct which compresses the ascending aorta leading to coarctation. The other mechanism is haploinsufficiency of X chromosomal genes like *CASK* and *USP9X* which are important in regulating TGF-β-SMAD signalling pathway [12,13]. This leads to altered migration of neural crest cells into vascular smooth muscle and altered regulation of matrix proteins causing defective valve formation and root dilatation. It might be that the former mechanism is more important for coarctation, which is therefore more prevalent in classic TS who are more prone to in-utero lymphedema formation. The second mechanism probably explains the valvular/septal abnormalities. We also found a significantly higher aortic root diameter in classic TS. Developmental defects of the kidney but not collecting duct anomalies were higher in classic TS. Whether factors like obstruction of the tract of ascent of kidneys by distended lymphatic sac has any role to play in this is unknown.

The higher prevalence of conductive hearing loss in isochromosomes and classic Turner syndrome is explained by the fact that both these groups have a haploinsufficiency of Xp specific genes like *SHOX* which is expressed in the first two branchial arches. This leads to altered eustachian tube mechanics and abnormal shape of the palate which predispose to fluid accumulation in the ear leading to secondary infections and conductive hearing loss.

Though the prevalence of anti-thyroid antibodies was similar in all groups, an increased severity of autoimmune responses of the thyroid could explain an earlier onset and hence higher prevalence of overt hypothyroidism in isochromosomes. Interestingly, in a UK-based TS registry including adult TS only, isochromosomes had a lower prevalence of hypo-thyroidism whereas in an Iranian study, hypothyroidism was high among 45,X/46,XX and mosaic isochromosomes [3,14].

Almost all the girls tested had low PIQ. 45,X/46,XY mosaics had the highest and 45,X/46,XX mosaics the lowest VIQ, PIQ and arithmetic scores. Ours is the only Indian study on IQ in TS girls.There have not been many systematic studies on separate VIQ and PIQ assessment across different karyotypic variants in TS and age-appropriate battery of tests were not used. Individuals with TS are known to have an increased risk of selective impairment of non-verbal skills and performance IQ. A review by Ross, et al. [15] suggests that apart from hypoestrogenism, haploinsufficiency of genes on the short arm of the X chromosome (Xp) could be responsible for the hallmark features of the TS cognitive phenotype.

A limitation of our study was that cardiac MRI was not done to measure aortic size index. For celiac disease, we analyzed only anti-Ttg IgA and total IgA and did not

detect any positive case. The possibility exists that duodenal biopsies would have yielded more cases.

In developing nations like India, frequent monitoring for all comorbidities in Turner syndrome is difficult. Echocardiographic screening for congenital cardiac malformations should be done for all, but cardiac MRI with aortic size index estimation is a must for classic Turner syndrome patients, who have the highest prevalence of aortic coarctation and the largest aortic root diameter. IQ (verbal and performance) testing is most important for 45,X/46,XX mosaics. Similarly, frequent pure tone audiometry is most essential in the classic and isochromo-some Xq group. While annual thyroid function testing is recommended for all karyotypes of Turner syndrome, more frequent monitoring for overt hypothy-roidism may be necessary for Turner syndrome with isochromosome-Xq. We do not intend to underemphasize the importance of following existing guidelines for screening [20], but we suggest that our findings may help prioritize the most essential investigations in different karyotype groups.

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