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Original Article

A qPCR-based algorithm for the diagnosis of classic and non-classic Turner syndrome

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Background and objectives: Turner syndrome is an X-chromosome aneuploidy which include classic monosomy-X and variants like mosaicism, isochromosome-Xq, *etc*. It is diagnosed by karyotyping, which is time-staking, laborious and costly. Quantitative real-time PCR (qPCR) offers a faster and cheaper alternative testing strategy, but single- or dual-primer qPCR may miss some variants. This proof-of-concept study was conducted to evaluate multi-primer qPCR in detecting various karyotypes of Turner syndrome.

***Methods*:** Genomic DNA was extracted from 50 cases with Turner syndrome (45,X=23; 45,X/46, XX=10; isochromosome-Xq=12; 45, X/46, XY=5), 25 control females (46,XX), and 5 males (46,XY). DNA was analysed using fast qPCR with 4 primers targeting Xp-genes (*SHOX*, *ARSE*) and Xq-genes (*VAMP7*, *XIST*). The ΔΔCT method calculated gene dose relative to 46,XX females, with *HBB* being the housekeeping gene. Gene cut-offs were ascertained by receiver –operator-curve (ROC) analysis. This was followed by developing an algorithm for detecting classical and non-classical Turner syndrome.

Results: Using the criteria *SHOX* <0.752 "OR" *ARSE* <0.885, all the cases of Turner syndrome were detected with 100% sensitivity and 93.3% specificity. *VAMP7* >0.723 detected isochromosome-Xq- Turner syndrome with 87.9% sensitivity, and 72.7% specificity. SHOX < 0.511 differentiated classic Turner syndrome from 45,X/46,XX mosaics with a 70% sensitivity, and 78.3% specificity. Our qPCR-based algorithm showed near-perfect agreement (Cohen's *k*=0.81) with 100-cell karyotyping, identifying 14 of 15 Turner syndrome cases with low-level mosaicism missed by 30-cell-karyotyping.

Interpretation and conclusions: A qPCR-based algorithm can be used for the rapid detection of classic and non-classic Turner syndrome, pending further validation studies. However, it cannot detect ring-chromosomes, mosaic-polyploidy and is inadequate to pinpoint the karyotypic subtype of Turner syndrome.

Keywords Isochromosomes; Monosomy-X; qPCR; *SHOX*; Turner Syndrome; *VAMP7*

Turner syndrome is a common aneuploidy in girls caused by either X-monosomy (45,X or classic Turner syndrome), mosaicism of 45, X with other cell lines or different structural aberrations of X, like ring-chromosome or isochromosome-Xq.[1,2]

The gold standard test for diagnosing Turner syndrome is karyotyping from peripheral blood, which requires cells to be in metaphase arrest, and therefore has a turn-around time of 2–3 weeks. Its advantages include lower cost (~2,500 – 4,500/- INR per sample) and the ability to detect structural aberrations which might be missed on other cytogenetic methods involving region specific probes.[3-5] Rapid molecular diagnostic methods to detect aneuploidies are fast becoming popular, given their high throughput nature, lower cost, lesser propensity to errors and the ability to

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yield results within hours. These include fluorescence in-situ-hybridisation (FISH), multiplex ligation dependent probe amplification assay (MLPA) and quantitative fluorescent-PCR (QF-PCR) and but most of these require expensive equipment.[6-9] FISH can be conducted during interphase reducing turn-around time to 1 – 3 days, but diagnosing the different variants of Turner syndrome would require multi-probe FISH (multiple probes on different parts of the X and also Y chromosome) or possibly whole chromosome painting, increasing the average cost to 8000 – 22000/- INR per sample.[10,11] QF-PCR and qPCR are two automation-friendly, rapid assays with costs between 2500 – 4500/- INR per sample. Currently, QF-PCR is commercially used for prenatal aneuploidy detection and qPCR for viral-load estimation. QF-PCR provides results within 1 – 3 days and qPCR in a few hours. One main problem with QF-PCR is that its interpretation depends on the relative dosage calculation from peak ratios (allelic quantification, not absolute copy number); a single peak where two are expected could suggest monosomy or homozygosity. This makes it difficult to detect monosomy involving the X chromosome and it could also miss low or moderate level mosaicism.[8,12] These make qPCR an interesting and inexpensive tool for large scale aneuploidy detection like in neonatal screening for Turner syndrome or large scale testing among all girls with short stature. Real-time quantitative PCR was used in a few small studies to diagnose Turner syndrome using different X-chromosome-markers.[13-16] In most studies, the sensitivity for the detection of non-classic variants of Turner syndrome was low.

A 30-cell karyotype can sometimes yield erroneous or false negative results in girls who have a high clinical suspicion for Turner syndrome. Often, their diagnosis of Turner syndrome is made by 100 cell-karyotyping or gonadal-tissue karyotyping. This is further time consuming and laborious. Conceptually, if a PCR based diagnostic test is designed using 3 or 4 different primers on different locations of the X-chromosome on its p and q arms and used along with a gene on the Y chromosome and autosomal house-keeping genes, it should detect almost all forms of Turner syndrome as it involves either total or partial loss of the X chromosome or a mosaicism of these cells along with normal 46,XX or 46,XY cells, or large deletions of the X-p or duplication of the X-q.

This proof-of-concept study aimed to develop and validate a qPCR-based diagnostic algorithm for detecting karyotypic variants of Turner syndrome.

Table I. Number of samples with different karyotypes selected for the study

Karyotype	N = 80
Classic Turner syndrome (45,X)	23
Mosaic Turner syndrome (45,X/46,XX)	10
Turner syndrome with isochromosome Xq	12
45,X/46,XY	5
46,XX (normal female controls)	24
46,XY (normal male controls)	6

Methods

This study was undertaken together by the department of Endocrinology and Metabolism, IPGME&R and department of Endocrinology, NRS Medical College, Kolkata, West Bengal, India. Ethical clearance for this study was obtained from the institutional ethics committees of both the participating institutes.

Study participants: We included 50 cases with karyotype-proven-diagnosis of Turner syndrome , 24 karyotype-normal females (46, XX), and six karyotype-normal males (46, XY) by convenience sampling from the Endocrinology outpatient departments of two tertiary care hospitals. The karyotype distribution of the samples is outlined in **Table I**. Sample size calculation was not performed since this was a proof-of-concept study for a rare disorder. The sample size was limited but considered adequate to establish technical feasibility and preliminary diagnostic accuracy.

Choice and design of primers: The primers used were short-stature-homeobox gene (SHOX) present on the pseudo-autosomal-region (PAR)1 of Xp and Yp (Xp22.33 and Yp 11.2), Vesicle-associated-membrane-protein-7(VAMP7) on PAR2 of Xq and Yq(Xq28 and Yq12), Aryl-sulfatase-E (ARSE) gene on non-pseudo-autosomal region of Xp(Xp22.3) and X-inactive-specific-transcript(XIST) on non-pseudo-autosomal region of Xq(Xq 13).

The rationale behind the choice of these four primers was that choosing genes in both short and long arms of X will aid in detecting not only complete haplo insufficiency of X as seen in classic-Turner syndrome (45,X) but also detection of isochromosome-Xq wherein genes on Xp are supposed to be halved and those on Xq are supposed to be 1.5 times that of 46,XX females, as well as detection of structural aberrations like large deletions of Xp. A Y-chromosome primer SRY (Yp11.2) was assessed by qualitative PCR to detect 46,XY and 45,X/46,XY

samples. HBB (11p15.5) was an autosomal gene used as a housekeeping gene to neutralise any errors arising due to differences in DNA amounts in the cases and control samples. Primers for amplification of specific genes were designed using the Primer 3 software (*https://primer3plus.com/*) (**Supplementary Table I**). Specificity of the primers was confirmed using a single melting curve peak (**Supplementary Fig. 1**). Several primers were tested with different concentrations of DNA templates from 0.01 ng/mL to 10 ng/mL to obtain standard curves. Primers with the best standard curves (least primer-primer dimer formation and with a sharp CT value peak) and the combination of primers with the closest primer efficiencies were chosen for further experiments. **Supplementary Table II** summarises the expected results of gene doses of different primers on qPCR for the different karyotypes

Sample collection and PCR procedure: About 2 mL of peripheral blood was drawn from the patients and controls following consent. Genomic DNA was isolated using DNA isolation and purification kit QIAamp DNA Mini Kit (QIAGEN, USA). Purity and concentration of DNA was determined using spectrophotometer. Samples were stored at -40°C till further use. PCR amplification and detection were carried out using the Applied Biosystems™ 7500 fast real-time PCR system v2.0 (Applied Biosystems, USA) in 96-well plates (Fast) using SYBR green MasterMix from Thermo Scientific (Thermo Fisher, USA).

Twenty microlitre (20 µL) reaction volume was set up with 10 µL MasterMix with ROX (2X stock), 0. 5 µL of each forward and reverse primer, 2 µL DNA template (amount of DNA) and 7 µL of ultrapure water. For each of the qPCR experiments, a DNA concentration of 2.5 ng/mL was taken. Target gene dose calculation was done by the comparative or ΔΔCt method.[9] A fixed set of three references were run for each of the experiment (to minimise errors) and their mean Ct values was considered as reference-Ct. This was then used to determine fold changes in Turner syndrome cases and controls using the formula $2^{(\Delta\Delta Ct)}$. All samples were run in duplicate and discordant Ct values (>5% difference in Ct values between the two technical duplicates) were not accepted for analysis. Mean ΔCt of acceptable samples were used for gene dose calculation.

Study period: The study was performed in two phases, from January 2018 to December 2020 and from May 2025 to August 2025.

Comparison with controls and designing of algorithm: Results of gene doses of the Turner syndrome -samples were compared with those of normal females and between Turner syndrome -karyotypes. Using the cut-offs for the different primers derived from ROC curves, we proposed an algorithm for the diagnosis of all forms of Turner syndrome. The proposed qPCR-based diagnostic algorithm is based on internal results. We also analysed samples from an additional 15 girls who had a clinical suspicion of Turner syndrome but were found to be 46, XX on 30 cell-karyotyping. Using our algorithm, we made a diagnosis of Turner syndrome and the possible underlying karyotype in these cases. We compared our diagnosis with the results of 100-cell karyotyping in these girls.

Statistical methods: Statistical analysis were done using GraphPad Prism software version 6e for Mac. ANOVA or equivalent non-parametric test (Kruskal Wallis test) followed by post-hoc Tukey's multiple comparison tests were done to compare median values for the primers among the different karyotype groups. Unpaired t-test was used wherever two groups were compared. ROC curves were analysed to obtain the cut-offs with best sensitivity and specificity using Youden's J statistic. Cohen's K was used to analyse agreement between 100-cell karyotyping and qPCR-based diagnosis. $P<0.05$ was considered significant.

Results

Quantification of gene doses across different karyotypes of Turner syndrome: The proportion of different karyotypes of Turner syndrome patients and female and male controls is outlined in **Table I**.

Among the karyotypes, there were significant differences between medians for the *SHOX*, *VAMP7* and *ARSE* genes but not the XIST gene on ANOVA ($P<0.001$) (**Table II**). The wide range for gene-doses in the isochromosome-Xq group was due to mosaic-isochromosomes (45, X/46,X,iXq) and variable location of the breakpoint region on Xq. One sample had a karyotype of 45,X/46,X,idic(X)p and therefore >1.5 times the expected gene-dose for SHOX. Individual karyotypes of the 27 samples with non-classic variants of Turner syndrome with their results of qPCR of the different genes are outlined in **Table III**. The outliers among 45,X/46,XY mosaics can be explained by two twins with 45,X/46,X,idicYp mosaicism who had greater-than-expected results with Yp (PAR1) primer SHOX and lesser-than-expected values with Yq (PAR2) primer *VAMP7*. The SHOX gene dose had good correlation with the percentage of cells with 46,XX among the 45,X/46,XX mosaics (Spearman's r =0.93, $P<0.001$).

Table II. Results of qPCR derived gene doses for SHOX, ARSE and VAMP7 primers in the different karyotype groups of Turner syndrome, control females and control males

Karyotype of samples	*SHOX* gene; Mean (SD)	*ARSE* gene; Mean (SD)	*VAMP7* gene; Mean (SD)
Control females (46,XX)	1.05 (0.29)	1.08 (0.21)	1.09 (0.30)
Classic Turner syndrome (45,X)	0.45 (0.09)	0.75 (0.19)	0.49 (0.11)
Mosaic Turner syndrome (45,X/46,XX)	0.59 (0.15)	0.70 (0.12)	0.62 (0.16)
Turner syndrome with isochromosome Xq	0.56 (0.40)	0.69 (0.13)	0.78 (0.48)
Control males(46,XY)	1.08 (0.23)	1.03 (0.14)	1.05 (0.18)
45,X/46,XY mosaic Turner syndrome	0.79 (0.3)	0.54 (0.06)	0.51 (0.02)

SHOX is present on pseudo autosomal region(PAR1) of Xp and Yp, *VAMP7* is present on pseudoautosomal region PAR2 of Xq and Yq, *ARSE* is present on non-PAR region of Xp and XIST on non-PAR region of Xq

Table III. Distribution of different karyotypes with non-classic Turner syndrome variants and their respective gene doses for the four primers (SHOX and ARSE on Xp, VAMP7 and XIST on Xq)

	Doses for the different genes on qPCR			
Karyotype distribution	*SHOX**	*ARSE**	*VAMP7**	*XIST**
45,X[40]/46,XX[60]	0.604	0.497	0.629	0.574
45,X[60]/46,XX[40]	0.441	0.688	0.638	0.697
45,X[70]/46,XX[30]	0.518	0.747	0.719	0.807
45,X[55]/46,XX[45]	0.479	0.728	0.442	0.583
45,X[72]/46,XX[28]	0.428	0.946	0.456	0.834
45,X[34]/46,XX[66]	0.634	0.580	0.775	0.612
45,X[32]/46,XX[68]	0.867	0.733	0.353	0.657
45,X[45]/46,XX[55]	0.577	0.754	0.602	0.888
45,X[33]/46,XX[67]	0.835	0.754	0.753	0.657
45,X[50]/46,XX[50]	0.514	0.591	0.862	0.475
46,X,iXq	0.318	0.928	1.011	0.659
46,X,iXq	0.490	0.499	0.998	0.792
46,X,iXq	0.376	0.637	1.961	0.719
46,X,iXq	0.208	0.801	1.077	0.668
46,X,iXq	0.292	0.754	1.705	0.642
46,X,iXq	0.260	0.657	0.735	0.495
45,X[22]/46,X,iXq[88]	0.358	0.543	0.535	0.779
45,X[55.5]/46,X,iXq[44.5]	0.590	0.782	0.727	1.084
45,X[24]/46,X,iXq[76]	0.771	0.723	0.406	1.08
45,X[36]/46,X,iXq[64]	0.823	0.694	0.832	0.89
45,X[60]/46,X,iXq[40]	0.616	0.616	0.527	0.801
45,X[45]/46,idic(X)p[40]	1.695	0.796	0.553	0.781
45,X/46,XY	0.406	0.585	0.799	0.508
45,X/46,XY	0.612	0.583	0.531	0.661
45,X/46,X,idic(Y)p	1.071	0.476	1.094	0.477
45,X/46,X,idic(Y)p	1.105	0.465	0.962	0.599
45,X/46,XY	0.790	0.578	0.542	0.485

**SHOX* is present on pseudo autosomal region (PAR1) of Xp and Yp, *VAMP7* is present on pseudoautosomal region PAR2 of Xq and Yq, *ARSE* is present on non-PAR region of Xp and XIST on non-PAR region of Xq

ROC curve analysis: ROC curves were used to analyse the sensitivity and specificity at different cut-offs for the SHOX and ARSE primers on Xp in distinguishing

Table IV. Sensitivity and no of missed cases of turner syndrome by applying cut-offs of qPCR derived gene doses for Xp primers

qPCR result with Xp primers	Sensitivity (%) of the gene cut-off for the detection of different variants of Turner syndrome			No of missed cases
	Turner syndrome (All karyotypes) (n=50)	Classic Turner syndrome (45,X) (n=23)	Non-classic Turner syndrome variants (n=27)	
SHOX < 0.752	89.4	100	79.2	5
ARSE < 0.885	83.4	87.4	92.6	8
Either SHOX <0.752 or ARSE <0.885	100	100	100	0

Turner syndrome from control females (46,XX), outlined in the **Supplementary Figure 2**. For *SHOX* gene, AUROC curve was 97.2% (P<0.001) and a cut-off of 0.752 detected all Turner syndrome -karyotypes with a sensitivity of 89.4% and specificity of 89.7%. At this cut-off, all classic- Turner syndrome were detected and there were three false positives and five false negatives (all non-classic Turner syndrome, 45,X/46,XX=2, 45,X/46,X,iXq=2, 45,X/46,XY=1).

ARSE gene had AUROC of 93.1% (P<0.0001), and a cut-off of 0.885 had sensitivity of 83.4% and specificity of 82.6% for detecting Turner syndrome, with four false positives and eight false-negatives (45,X=6; 45,X/46,XX=1; 46,X,iXq=1) but all 45,X/46,XY mosaics were detected.

Algorithm to detect Turner syndrome and its variants using qPCR: If the criterion for a positive result was set as any one of the Xp primers (either *SHOX* or *ARSE*) less than the respective cut-offs, no case of Turner syndrome was missed, corresponding to a sensitivity of 100% (**Table IV**). The number of false positives was two out of the twenty-four samples of control females with 46,XX karyotype (8.33%).

ROC curves to determine cut-offs for gene doses to identify karyotypic variants of Turner syndrome: Between classic- Turner syndrome and 45,X/46,XX-mosaic-Turner syndrome, *SHOX* dose differed significantly (median 0.476 vs 0547, P=0.0008) and a cut-off of 0.511 distinguished classic Turner syndrome from mosaics with sensitivity 70% and specificity 78.3%.

VAMP7 gene on Xq showed significant differences between isochromosome-Xq vs other Turner syndrome (0.832 *vs.* 0.502, respectively; P<0.001). However, no such differences were noted using the *XIST* gene. ROC curve for *VAMP7* had an AUC 83.8% (P<0.001) and a cut-off of 0.723 detected isochromosome-Xq with 87.9% sensitivity and 72.7% specificity. At this cut-off, all pure isochromosomes (46,X,iXq) could be detected, but 2 out of 5 cases with mosaic-isochromosomes (45,X/46,X,iXq) were missed. Among Turner syndrome with other karyotypes, one case of classic Turner syndrome and three cases with 45,X/46,XX mosaics were falsely detected as isochromosomes using the *VAMP7* cut-off criterion, therefore leading to a false positivity rate of 10.5%.

Detection of 45,X/46,XY mosaics: Qualitative-PCR to detect SRY gene in 45,X/46,XY mosaics found positive bands for all five 45,X/46,XY mosaics in our cohort.

qPCR- based diagnosis in Turner syndrome cases with dubious karyotypes/low level mosaicism: We included an additional fifteen cases with a phenotypic diagnosis of Turner syndrome whose 30-cell karyotype was 46,XX (n =12) or 46,XY (n = 3). Using our algorithm, we found that the qPCR-based diagnosis for eleven of these twelve girls having 46,XX on 30-cell-karyotype was 45,X/46,X,iXq (n = 5) or 45,X/46,XX (n = 6). In all the three girls with 46,XY on 30-cell-karyotype, the qPCR-based diagnosis was 45,X/46,XY. We repeated a karyotype of 100 cells for these cases and found that the qPCR-based diagnosis matches with 100-cell-karyotype result in 14 out of the 15 (93.33%) cases. One patient with a diagnosis of 45,X[20]/46,XX[70]/46,X,iXq[10] could not be detected by qPCR-based-algorithm. The Cohen's kappa between qPCR-algorithm and 100-cell-karyotype was 0.81 (SE: 0.111, p andlt;0.001), indicating a near perfect agreement.

Discussion

In the present study, we used qPCR to determine doses of four genes on Xp and Xq and tested the performance of qPCR in diagnosing different cytogenetic variants of Turner syndrome, considering karyotype as the gold standard. We found that qPCR using a combination of two Xp primers- *SHOX* and *ARSE* detected all cases of Turner syndrome, serving as a good screening test for Turner syndrome. However, the false positive rate was ~8%, mandating a karyotypic confirmation in qPCR positive cases. Among subjects with Turner syndrome, qPCR using the Xq-gene *VAMP7* identified

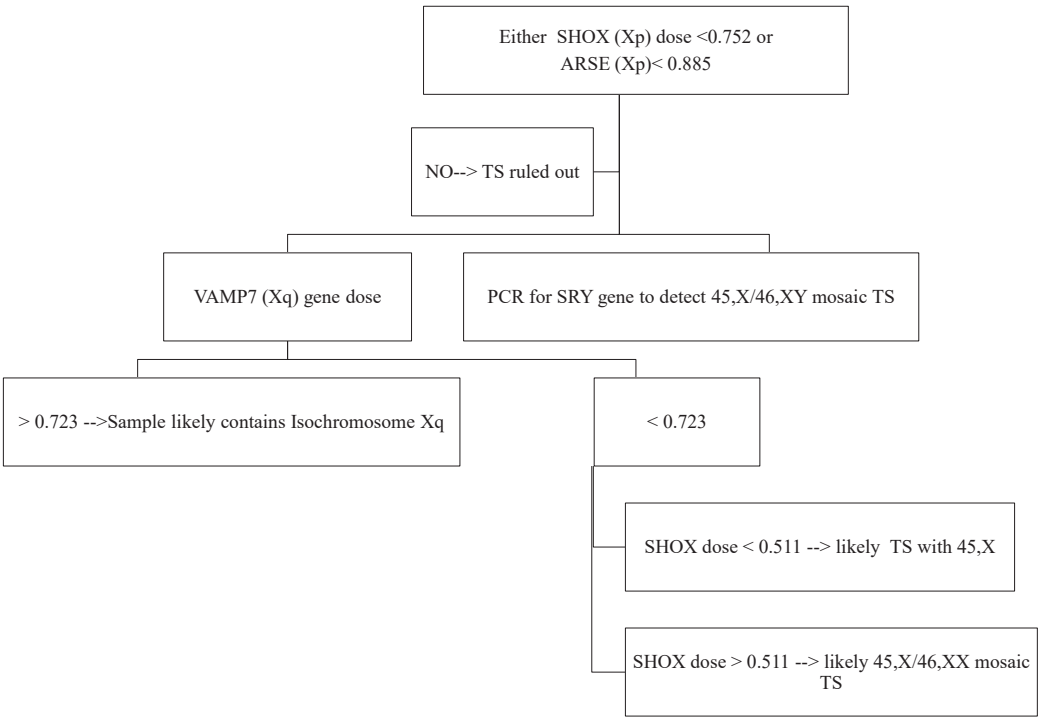

Figure. Algorithm for screening of Turner Syndrome using different primers by qPCR and its proposed application in diagnosing the different karyotypic variants of Turner syndrome.

all the isochromosome-Xq and most cases of mosaic isochromosome Xq. Based on our internal results, we propose a hierarchal form of testing for Turner syndrome, with *SHOX* and *ARSE* genes being used for the initial screening test. The screened positive cases should then be interrogated with the *VAMP7* gene to detect isochromosome-Xq and the *SRY* gene to detect 45,X/46,XY mosaicism. The *SHOX* gene dose could distinguish classic Turner syndrome from mosaic Turner syndrome, but the sensitivity and specificity were poor. Prior similar studies have focused on single Xp or Xq specific primers.[13-15] Some recent studies have used multiplex PCR to detect presence of Y-chromosomes in Turner syndrome .[16] Our cut offs are close to the results of the other studies and the sensitivity and specificity for detecting non-classic Turner syndrome are better than prior studies.[14,15] Furthermore, we could detect all the cases of Turner syndrome by qPCR using two Xp primers with an "either/or" criteria and designed an algorithm of hierarchical testing (**Figure**) using which most non-classic variants of Turner syndrome can be identified.

The diagnosis of Turner syndrome is routinely done by karyotyping of 20 to 30 peripheral blood cells. However, sometimes despite having a strong clinical suspicion of Turner syndrome, cases of mosaicism of 45,X cell lines with other cell lines may be missed, particularly if the proportion of 45, X cell lines is very low. In such cases, karyotyping of 100 cells or of buccal or gonadal tissue is indicated for diagnosing Turner syndrome. Similarly, cases of 45,X/46,XY Turner syndrome may sometimes be missed on routine karyotyping. Phenotypic females with 46,XY karyotype can be cases with testicular dysgenesis or complete androgen insensitivity; while those with 45,X/46,XY karyotype are either mixed gonadal dysgenesis or Y chromosome bearing Turner syndrome. It is very important to differentiate this latter group from pure 46,XY gonadal dysgenesis since those with 45,X/46,XY karyotypes need screening for all comorbidities of Turner syndrome, merit growth hormone therapy and need early gonadectomy for risk of gonadal tumours.[1,17] Our qPCR based diagnostic algorithm was able to detect these Turner syndrome cases with low level mosaicism of 45, X cell lines with 46,XX or 46,XY cell lines and might supervene the need for a 100 cell or gonadal tissue karyotyping in such cases.

Our study had the limitations of a small sample size and needs verification in further larger studies. A limitation of q-PCR as a technique is its inability to detect mosaics with X-polyploidy like 45,X/46,XXX and ring chromosomes, marker chromosomes, and structural defects of the X chromosome involving

शोध-संदेश

टर्नर सिंड्रोम (TS) 'x' क्रोमोसोम की कमी से होने वाली एक जन्मजात बीमारी है। यह अध्ययन टर्नर सिंड्रोम के शीघ्र और सटीक निदान से संबंधित है। इसमें qPCR आधारित एक एल्गोरिथ्म के माध्यम से क्लासिक एवं नॉन-क्लासिक TS की पहचान करने की संभावना का मूल्यांकन किया गया। अध्ययन से पता चला कि qPCR आधारित यह विधि TS के विभिन्न प्रकारों की तेजी से पहचान करने में सहायक हो सकती है और पारंपरिक कैरियोटाइपिंग (karyoptyping) की तुलना में सरल एवं कम समय लेने वाली है। हालांकि, यह तकनीक कुछ विशेष प्रकारों जैसे रिंग-क्रोमोसोम एवं मोज़ेक-पॉलीप्लॉइडी की पहचान करने में सक्षम नहीं है और TS के सटीक उपप्रकार को निर्धारित करने में सीमित है। इसलिए इसके व्यापक उपयोग से पहले और अधिक शोध एवं सत्यापन की आवश्यकता है।

deletions proximal to Xp24, unless it involves partial or complete deletion of one of the primers used for qPCR. However, they constitute an extremely small proportion of Turner syndrome and in this study, we had excluded such samples. Notably, there were two false positive cases detected by our algorithm. First, since this is a proposed screening test, we used the "OR" criteria for *SHOX* and *ARSE* genes on Xp so that none of the cases were missed. Since our cohort had a substantial proportion of mosaic Turner syndrome in addition to classic Turner syndrome, the *ARSE* gene dose had a wide range. The median *ARSE* dose for Turner syndrome was much higher than the expected value of 0.5 with a wide variation, the possible explanation might be the presence of *ARSE* pseudogene on Y.[18] Two patients had isodicentric Yp [45,X/46,X,idicYp] and the two copies of Yp might contribute to a higher median value of ARSE. Thus, a higher *ARSE* cut-off and using the OR criteria to avoid false negatives might have led to the false positives. Hence, we suggest this as a screening test, with positive results needing karyotyping confirmation.

We studied children, adolescents, and adult women with Turner syndrome (age range: 5 to 32 years). While we did not include neonates in our study, a similar strategy has been used successfully for neonatal screening.[14-16] DNA-based qPCR results are expected to be stable from neonatal period to adulthood. Also, we used an age-stable reference gene *HBB* and gene-doses were calculated using the comparative ΔΔCt method using healthy controls of similar age. In advanced age, there is a possibility of age dependent changes in DNA integrity, mostly affecting telomere length and methylation sensitive regions. We are not sure about how the results might change in the elderly with Turner syndrome. It may not be applicable to antenatal aneuploidy detection due to possible contamination with maternal cells.

Our results suggest that qPCR can be a reasonably accurate and easy-to-perform screening test for classic and non-classic Turner syndrome. Using multiple Xp, Xq and Y chromosome primers, our qPCR-based algorithm could detect cases of Turner syndrome that were not diagnosed with 30-cell karyotyping but only with 100-cell karyotyping. We propose that qPCR may be used as a cheaper but reasonably accurate alternative to the time and labour-staking process of karyotyping, pending further studies with larger sample size.

Author contributions: CB: Conducting experiments, analysis, data interpretation; SM: Acquisition of data, analysis of raw data with interpretation, conduct of qPCR experiments, manuscript writing; CS: Conducting experiments, analysis, data interpretation; NS: Supervision of data correctness, manuscript writing; NB: design of project, data analysis; Satinath Mukhopadhyay: Supervision of data correctness, manuscript writing. All authors have read and approve the final printed version of the manuscript.

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References

1. Gravholt CH, Viuff MH, Brun S, Stochholm K, Andersen NH. Turner syndrome: Mechanisms and management. *Nat Rev Endocrinol*. 2019;15:601–14.
2. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, *et al*. Clinical practice guidelines for the care of girls and women with turner syndrome: Proceedings from the 2016 Cincinnati international turner syndrome meeting. *Eur J Endocrinol*. 2017;177:G1–G70.
3. Fiot E, Alauze B, Donadille B, Samara-Boustani D, Houang M, De Filippo G, *et al*. Turner syndrome: French national diagnosis and care protocol (NDCP; National diagnosis and care protocol). *Orphanet J Rare Dis*. 2022;17:261.
4. Ghosh K, Appaswamy G, Jayakrishna T. Quantitative fluorescent PCR (QF-PCR) for prenatal diagnosis of fetal aneuploidy: Indian experience. *Indian J Obstet Gynecol Res.* 2018;5:419–23.
5. Zhang H, Xu Z, Chen Q, Chen H, Ding X, Liu L, *et al*. Comparison of the combined use of CNV-seq and karyotyping or QF-PCR in prenatal diagnosis: A retrospective study. *Sci Rep*. 2023;13:1862.

6. Sarikaya E. Is it time to start newborn screening for turner syndrome? *JSM Genet Genomics*. 2017;4:1023.
7. Banu M, Pathan AAK, Chaitanya K.V. Diagnostics for genetically inherited disorders: From cytogenetics to genomics technologies-A review. *Biomed Pharmacol J*. 2023;16:639–51.
8. Zhang H, Xu Z, Chen Q, Chen H, Ding X, Liu L, *et al*. Comparison of the combined use of CNV-seq and karyotyping or QF-PCR in prenatal diagnosis: A retrospective study. *Sci Rep.* 2023;13:1862.
9. Rivkees SA, Hager K, Hosono S, Wise A, Li P, Rinder HM, *et al*. A highly sensitive, high-throughput assay for the detection of Turner syndrome. *J Clin Endocrinol Metab.* 2011;96:699–705.
10. Veselinyová D, Mašlanková J, Kalinová K, Mičková H, Mareková M, Rabajdová M. Selected insitu hybridization methods: Principles and application. *Molecules.* 2021;26:3874.
11. Abulhasan SJ, Tayel SM, Al-Awadi SA. Mosaic Turner syndrome: Cytogenetics versus FISH. *Ann Hum Genet*.1999;63:199–206.
12. Cottino L, Sahibdeen V, Mudau M, Lekgate N, Krause A. QF-PCR: A valuable first-line prenatal and postnatal test for common aneuploidies in South Africa. *J Community Genet.* 2022;13:355–63.
13. Ibarra-Ramírez M, Zamudio-Osuna MJ, Campos-Acevedo LD, Gallardo-Blanco HL, Cerda-Flores RM, Rodríguez-Sánchez IP, *et al*. Detection of Turner syndrome by quantitative PCR of SHOX and VAMP7 genes. *Genet Test Mol Biomarkers*. 2015;19:88–92.
14. Corrêa SC, Rocha MN, Richeti F, Kochi C, Silva E Lima LA, Magalhães M, *et al*. Neonatal detection of turnersyndrome by real-time PCR gene quantification of the ARSE and MAGEH1 genes. *Genet Mol Res.* 2014;13:9068–76.
15. Rocha MN, Longui CA, Kochi C, Corrêa CS, Faria CD, Richeti F, *et al*. Applicability of real-time PCR methodology in the neonatal detection of Turner syndrome. *Horm Metab Res*. 2010;42:677–81.
16. Campos-Acevedo LD, Ibarra-Ramirez M, de Jesús Lugo-Trampe J, de Jesús Zamudio-Osuna M, Torres-Muñoz I, Del Roble Velasco-Campos M, *et al*. Dosage of sex chromosomal genes in blood deposited on filter paper for neonatal screening of sex chromosome aneuploidy. *Genet Test Mol Biomarkers.*2016;20:786–90.
17. Zhao Q, Chen S, Sun H, Yang W, Ban B. A multiplex PCR-based sensitive and specific method for detecting Y chromosome material in patients with Turner syndrome. *Chin J Med Genet*. 2022;39:1216–23.
18. Meroni G, Franco B, Archidiacono N, Messali S, Andolfi G, Rocchi M, *et al*. Characterization of a cluster of sulfatase genes on Xp22.3 suggests gene duplications in an ancestral pseudoautosomal region. *Hum Mol Genet*. 1996;5:423–31.

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