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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*