

Detection of Turner Syndrome by Quantitative real time PCR of VAMP7 Genes

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Abstract

Turner Syndrome (TS) is an unfavorable genetic condition with a prevalence of 1:2500 in newborn girls. Prompt and effective diagnosis is very important to appropriately monitor the comorbidities. The aim of the present study was to propose a feasible and practical molecular diagnostic tool for newborn screening by quantifying the gene dosage of the VAMP7, XIST, UBA1, and SRY genes by quantitative polymerase chain reaction (qPCR) in individuals with a diagnosis of complete X monosomy, as well as those with TS variants, and then compare the results to controls without chromosomal abnormalities. According to our results, the most useful markers for these chromosomal variants were the genes found in the pseudoautosomal regions 1 and 2 (PAR1 and PAR2), because differences in gene dosage (relative quantification) between groups were more evident in VAMP7 gene expression. Therefore, we conclude that these markers are useful for early detection in aneuploidies involving sex chromosomes.

Keywords: constitutional growth delay, growth hormone deficiency, short stature

Introduction

Turner syndrome (TS) is a genetic disorder occurring in females caused by the partial or complete absence of one of the X chromosomes. The condition affects approximately 1 in every 2500 females and requires a chromosomal analysis for definite diagnosis [1]. Short stature and hypergonadotropic hypogonadism are the principal features of TS [2,3]. Patients with TS are also susceptible to numerous other medical conditions, such as endocrine and metabolic disorders, autoimmune disease, and cardiovascular disease [4]. Multiple karyotypes including 45, X haploinsufficiency, 45, X with mosaicism, or X chromosome anomalies are associated with variable presentations along the Phenotype spectrum; individuals with 45, X monosomy typically have the most severe phenotype [5]. Mosaic TS are subcategorized according to whether the second cell line contains a whole or part of a sex chromosome. In a study by Jacobs et al. [6], 16% of the 84 cases with TS had a standard karyotype of 45, X and a second cell line containing a ring chromosome X. The phenotypic variability of these mosaics is largely dependent on the size of the ring and the presence of a functioning *XIST*. Patients with TS tend to have short stature and high body mass indices [7], but most often do not have growth hormone (GH) deficiency [4]. Females with TS make GH naturally in the pituitary gland, but their bodies do not use it appropriately. GH provocation tests are

generally not indicated in TS unless the growth velocity is extremely low for the age and sex. Thus, the concurrent occurrence of GH deficiency and TS is a very rare condition. Moreover, the association of TS with hypopituitarism is also an uncommon finding [8]. To the best of our knowledge, there have been no previous reports of concomitant GH deficiency and structural pituitary abnormalities in TS. Here, we report the first case of the coexistence of GH deficiency and pituitary micro adenoma in a TS patient

The X chromosome has 155 mega base pairs (Mb) and contains 1000 genes (Bianchi *et al.*, 2012), while the Y chromosome (60 Mb) contains only 104 protein-coding sequences out of a total of over 200 genes (Li *et al.*, 2008).

Inactivation of the majority of one X chromosome in females leads to a functional 1n dosage of X-linked genes in both genders. The pseudo autosomal regions (PAR)1 and 2 homologous sequences, which are present on both the X and Y chromosomes, escape from X chromosome inactivation. Therefore, the 24 genes present in the 2.6 Mb PAR1 (Mang and Morris, 2007) and the 4 genes in the 320 kb PAR2 have a functional gene dosage of 2n in both genders. Hence, the etiology of TS lies in the haploinsufficiency of genes located in the PAR1 (Zhong and Layman, 2012).], two X-specific genes (ubiquitin-like modifier activating enzyme 1 [*UBAI*] in p, and X inactive-specific transcript [*XIST*] in q), the sex determining region Y (*SRY*) gene by qPCR in TS patients, and to compare the results to those for the gene dosage of men and women with a normal karyotype. These findings were used to evaluate the effectiveness of these measurements as an affordable method for TS screening.

Materials and Methods:

We performed a descriptive, comparative, no blinded study that included 30 Turner syndrome patients karyotyping. Between 2019 and 2020 who patient taken from gynecology department institute of medical science BHU. Subjects with a normal karyotype (15 females and 5 males). Were recruited from the same institution and used as controls. Written informed consent was obtained from all subjects or legal guardians, and the Health Research Ethics Board of the institute of Medical science BHU. Approved the study (Approval No.: GN-11-004). The subjects were divided into four groups according to their karyotype: (a) 45, X (X monosomy); (b) 46, XY (male); (c) 46, XX (female); and (d) TS variants (mosaicism and structural aberrations).

Sample collection

Biological blood samples. Four milliliters of peripheral venous blood was collected in a heparin-treated tube from each participant for G-banding karyotype in our laboratory. Only four patients and the control subjects were karyotyped during this study. Twenty patients who had been previously karyotyped and diagnosed with TS were invited to participate, and a 3-mL blood sample was collected in EDTA tubes to obtain total genomic DNA. Extraction was conducted using a salting-out method.

Gene selection. Two probe on-demand assays (Applied Bio red pcr system), were used to measure the dosage of five genes, *UBA1*, *XIST*, The *RNase P* gene was used as an autosomal reference control. Real-time PCR analysis. The dosage of each gene was measured with the bio red pcr System and TaqMan Genotyping Master Mix 2X following the manufacturer's instructions. Relative quantification (RQ) values were obtained using the Data CT comparison method. Amplification reactions were performed in triplicate with determined reproducibility. In TS patients, an $RQ < 1$ for *UBA1* and *XIST* was expected.

Statistical analysis

Descriptive statistics were applied to the RQ values of each gene. ANOVA was used to analyze intergroup differences between gene dosages, which were determined by the mean RQ values of each gene. Differences were considered statistically significant when the *p*-value was < 0.05.

Result:

Obtain samples, 40 were from the control group (10 46, XY and 10 46, XX) and 20 from TS patients. Among the latter, 4 (20%) had monosomy X and 4 had a TS variant, with 45,X/46,XX mosaicism being the most frequent (20%) karyotype (Table 1). In the case of the *UBAI* gene, the RQ in patients with complete X monosomy was from 0.49 to 0.58, and in the TS variant group, the RQ values ranged from 0.60 to 0.95. The control group RQ range for the same gene was from 0.99 to 1.00 for 46, XX and from 0.46 to 0.69 for 46, XY All of the gene markers in patients with different chromosomal variants were evaluated to analyze whether the chromosomal formula could be identified without a conventional karyotype. Using individuals with a 46, XX karyotype as a reference, a difference between the gene dose of *XIST* and *UBAI* was found; however, when compared to 46, XY individuals, who are hemizygous for these genes, the RQ was similar to that found in most patients.

Case	Karyotyping
1	45,X
2	45,X
3	45,X
4	45,X
5	45,X
6	45,X,inv (7)(q31.2-pter)
7	45,X[6]/46,XX[24]
8	45,X[2]/46,XX[28]
9	45,X[18]/46,XX[12]
10	46,XX
11	46,XX
12	46,XX
13	46,XX
14	46,XX
15	46,XX
16	46,XX
17	46,XX
18	46,XX
19	46,XX
20	46,XX

Table1: case and karyotyping result.

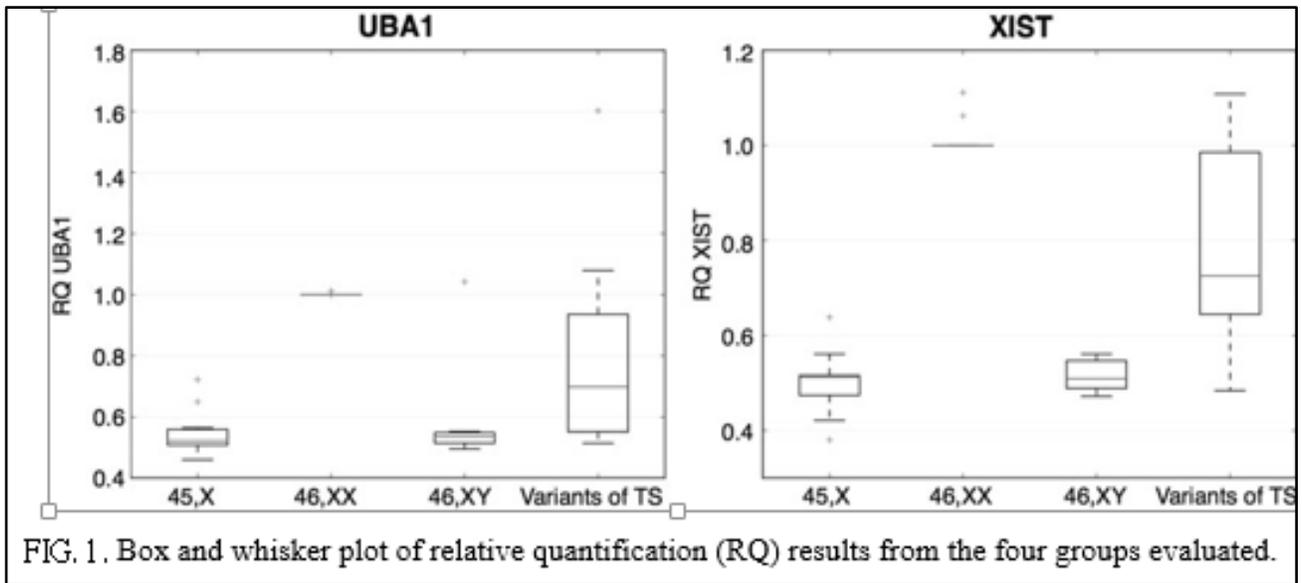
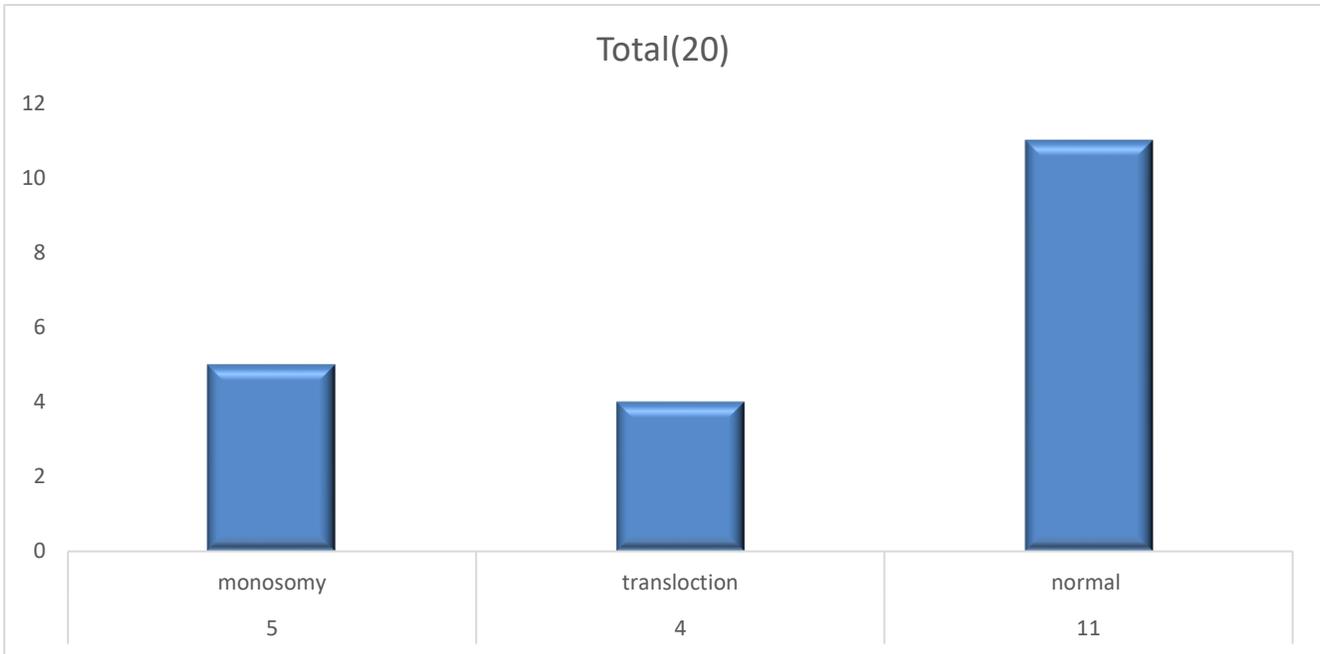


FIG. 1. Box and whisker plot of relative quantification (RQ) results from the four groups evaluated.

FIG. 1. Box and whisker plot of relative quantification (RQ) results from the four groups evaluated.

Discussion:

Early diagnosis of TS leads to proper treatment of complications and can aid in avoiding or reducing deleterious consequences in adult life. The frequent delay in the diagnosis of TS justifies the search for methodologies that allow prompt diagnosis of partial or complete, homogeneous or mosaic, and X chromosome monosomy. In the present study, we analyzed 20 patients with TS to determine the gene dosage of *XIST*, *UBA1*, in 20 individuals. The group with complete monosomy X represented 20% of all samples in the study group. Of the remaining TS patients, 20% were mosaic with 45,X/46,XX,

followed by other mosaics and structural aberrations present in similar proportions to those reported in the literature (Sybert and McCauley, 2004). In our study, the differences between RQ means in the patients with complete monosomy and control subjects were significantly different. Similar findings have been reported by Rocha *et al.* (2010). Overall, the patients had half of the dosage (compared to 46, XX women) in all X chromosome genes analyzed and the absence of the *SRY* gene. If the analysis of the studied genes is used as a screening test in a population, it will differentiate between individuals with a 46, XY karyotype and TS patients.

Conclusion:

The delay in TS diagnosis is a problem, and methodologies that allow early diagnosis of patients, including those with chromosomal variants, are needed. Management of patients with TS have addressed the potential benefit of designing a newborn screening method for the detection of these patients. We propose the quantification of the gene dose of as a screening tool for the diagnosis of newborns with TS.

Conflict of interest

Authors have declared that and research was conducted in and the absence of any commercial or financial relationships without any conflict of interest.

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Author Contributions

R.S., R., A. contributed to the conception, design, and writing of the study protocol and the design of search strategies; N.K.S., A K.Y., M.K. located and obtained reports, helped to select and assess cases, conducted the data analysis, and drafted and approved the final paper. All authors contributed to the conception, design, and writing of the study protocol, conducted data analysis and revised and approved the final paper.

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