

CYCLIN B1 EXPRESSION IN MOTHERS OF DOWN SYNDROME VERSUS EUPLOID CHILDREN

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Abstract.

Introduction. Down Syndrome (DS) is the most common chromosomopathy. Cyclin B1 is a regulatory protein involved in mitosis. **The aim was** to determine the cyclin B1 relative expression in mothers of DS patients. **Methods.** In this cross-sectional study, mononuclear cells of peripheral blood were obtained from 40 mothers of confirmed DS patients and 40 mothers of euploid patients, between 2019-2021. Demographic variables and cyclin B1 messenger RNA (mRNA) with real-time polymerase chain reaction (qPCR) were analyzed. The cases of DS mothers were compared to the relative expression of euploid group. **Results.** cyclin B1 mean relative expression by RT-PCR showed a significant difference between the group of mothers of DS children and mothers of euploid children ($p < 0.05$). The expression of the cyclin B1 was lower than the mean of mothers with euploid patients. Previously, it has been reported that the cyclin B1 down expression should accelerate telomere shortening in DS patients. Therefore, it would be advisable to recategorize the maternal age-related risk factor. **Conclusion.** Cyclin B1 may be a cost-effective biomarker in reproductive age women to present a DS case not associated with chronological age.

Keywords: biomarker, cyclin B1, Down Syndrome, risk factor.

Introduction

Down Syndrome (DS) is a genetic disorder in which cells present an additional copy of chromosome 21[1]. This syndrome is the most common type of chromosomal disorder among children. For example, in 2018, in Mexico, DS affected approximately one in every 689 live births[2]. Although nondisjunction in humans, which is associated with DS, has been associated with advanced maternal age, the adjacent process is unclear completely[3]. Typically, DS is characterized by cardiac and neurological alterations, but changes in cell signaling pathways associated with survival have also been described. Following this line of research, cyclins represent key components of the cell cycle regulation and have implications for the processes of tumorigenesis and proliferation. DS patients present a complex immune dysregulation induced by factors such as telomere damage, cellular senescence, mitochondrial dysfunction and an abnormal gene expression profile associated with the cell cycle that induces disease progression [4].

On the other hand, CDK1-cyclin B (Cyclin Dependent Kinase 1) is a member of the cyclin-dependent kinases necessary in eukaryotic cell cycle control to promote the G2-M pass. Cyclin B role in oocyte maturation and the embryo development process is significant. As such, CDK1 activation is required for M-phase. The metabolism of CDK1-cyclin B in oocytes and embryonic development is essential to the survival regulation and cellular checkpoint. Indeed, DS patients have altered G2-M permeability, indicating an intriguing unexplored target for disease progression disease[5].

Maternal cyclin B1 determination should provide a recategorized risk for women of reproductive age, to establish a cellular aging marker associated with oocyte maturation control and checkpoints of cell cycle progression[6–8]. It is well known that cyclin B1 is under-expressed in DS patients, but maternal cyclin B1 expression has been scarcely studied[9–11]. The objective of this study was to analyze the Cyclin B1 relative expression in maternal Mononuclear Cells of Peripheral Blood (MCPB) from mother who had or not had a baby with DS.

Methods:

Patients

Mothers tended at the "Mónica Pretelini Sáenz" Maternal Perinatal Hospital of the Health Institute of the State of Mexico (ISEM), Centro de Rehabilitación Infantil Teletón

(CRIT)-Guerrero, and Centro Estatal de Rehabilitación y Educación Especial (CEREE), Toluca, Mexico, between 2019 and 2021, were invited to this survey.

Two groups were studied: A) mothers of patients with trisomy 21, and B) mothers who had euploid patients (control group). Women had to be older than 18 years and without a background familiar chromosomopathy to be included in the study.

Anthropometry and Blood samples

To obtain the Body Mass Index (BMI), all participants were measured for height (m) and weight (kg). Blood samples were collected in a tube containing Ethylenediaminetetraacetic Acid (EDTA), placed on ice in a cooler, and all samples were transported to the laboratory and stored at -20°C until processing.

Total RNA extraction.

Total RNA extraction was performed following the protocol from Norgen Biotek Corporation, Canada (Cat. 17200, 37500, 17250). Explaining briefly, the total blood samples were treated with purification produced by binding RNA to column. A lysate was prepared with 100 µL of non-coagulated blood to an RNase-free microcentrifuge tube with 350 µL of RL buffer and 200 µL of ethanol. In an assembled column, 600 µL of lysate was transferred and centrifuged at 6,000 rpm for 1 minute within 2 minutes of lysate draw. Wash stages were applied with 400 µL of wash solution and centrifuged at 6,000 rpm for 1 and 2 minutes, three times each stage. Finally, the column was transferred to an RNA elution tube with 50 µL of elution solution and centrifuged at 14,000 rpm for 2 minutes. After RNA was extracted, samples were quantified and then stored at -70°C.

Quantitative real-time PCR.

For reverse transcription and quantitative real-time PCR (qPCR), the Sybr Green one-step RT-PCR kit (Thermo Scientific, USA) was used in a 7500 Fast Real Time PCR System (Applied Biosystems, Cheshire, UK). RNA previously extracted was unfrozen to confirm a A260/280 nm absorbance ratio > 1.8 (quality control). Total RNA concentration was calculated to determine the final volume with the NanoPhotometer (Implen GmbH, München, Germany). A final volume of 25 µL per reaction was calculated by mixing 0.4 mM of forward and reverse primers, and the final concentration of each gene was optimized with the Taguchi method.

The qPCR program included 30 min at 43°C, 2 min at 95°C; 40 cycles of 15 s at 95°C, 30 s at 55°C, followed by 30 s at 72°C. The primers' temperature for annealing was 63°C. To calculate fold amplification, the comparative threshold cycle (CT) method was utilized: $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT = (CT\text{-target} - CT\text{-reference})\text{ treated-sample} - (CT\text{-target} - CT\text{-reference})\text{ calibrator-sample}$. For the calibration, a quotient between the target gene normalized to the constitutive gene was applied to the expression level.

The primers used in the experiment were designed using the Gene Runner software v. 3.05 (Hastings Software, New York, USA) and synthesized at the Institute of Biotechnology, National Autonomous University of Mexico (UNAM) (Cuernavaca, Mexico).

A Basic Local Alignment Search Tool (BLAST) was applied to each primer sequence to assure hybridization to specific targets. The primers' sequences used were for glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward: 5-CTTTGGTATCGTGGAAGGACTC-3, reverse: 5-GTAGAGGCAGGGATGATGTTCT-3 and cyclin B1; forward: 5-AAT GAA ATT CAG GTT GTT GCA GGA G -3, reverse: 5-CAT GGC AGT GAC ACC AAC CAG -3.

Statistical analysis.

Quantitative variables were represented by measures of central tendency. First, the Kolmogorov test was performed to determine the normality of the variables. Student's T test or the Mann Whitney U test were used based on the Gaussian distribution of the variables. In all cases a $p \leq 0.05$ was considered statistically significant. The analyses were performed using SPSS v. 22 statistical software (IBM, Armonk, North Castle, New York, USA) and a $p\text{-value} \leq 0.05$ was considered statistically significant.

Ethics.

The study was approved by the Ethics Committee of the HMPMPS (Code: 13 CI/15/06 068/CONBIOÉTICA-15-CEI-005-20170615) and authorized by the Autonomous University of the State of Mexico (Code: DOCCSA-1020).

Results:

General data

40 mothers in each group were analyzed. The relative expression of cyclin B1 with a comparative analysis between mothers of Down's syndrome children and non-Down's

syndrome children is presented in Table 1.

Table-1

General data of the studied groups

Characteristic		Mothers of SD patients n=40	Mothers of euploid patients n=40
Age (años)		37.9	35.3
Weight (Kg)		58.9	62.5
Talla (cm)		153.4	152.5
BMI (Kg/m2)		21.7	20.9
Level of education	Primary education (%)	37.5	2.5
	Secondary education (%)	27.5	5.0
	Upper secondary, postsecondary non-tertiary or short-cycle tertiary (%)	17.5	42.5
	Bachelor's or equivalent level (%)	15.0	37.5
	Master's/Doctor or equivalent level (%)	2.5	12.5
Area	Urban (%)	85.0	97.5
	Rural (%)	15.0	2.5
Marital status	Married (%)	72.5	82.5
	Widowed (%)	2.5	0.0
	Separated (%)	15.0	0.0
	Divorced (%)	2.5	0.0
	Single (%)	7.5	17.5
Religious affiliations	Catholical (%)	85.0	97.5
	Non-Catholical (evangelical, Jehovah's witness) (%)	10.0	2.5
	Non-religious (%)	5.0	0.0

Cyclin B1 relative expression analysis

Upon analyzing the CT values of cyclin B1 expression, the differences between the two groups were statistically significant. The RU in the mothers of SD children were 15 and mothers of euploid children were 23.7, with statistical significance (15.0 vs. 23.7, respectively, p = 0.043) (Table 2) (Figure 1).

Table-2

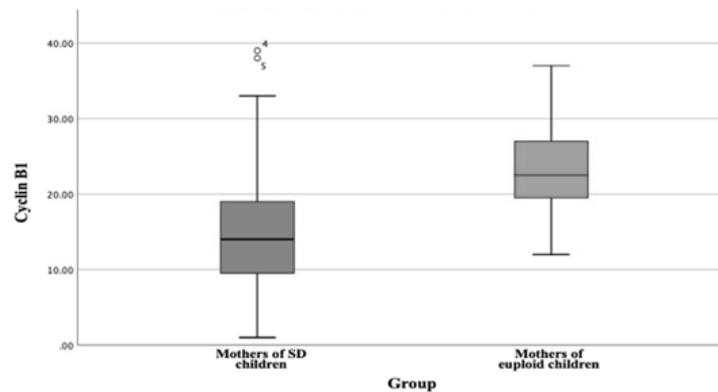
Cyclin B1 relative expression of the studied groups

Variable	Group	Mean (RU)	Standard deviation	2 -ΔΔCT	P-value
Cyclin B1	Mothers of SD children n=40	15	8.67061	1.37094	0.043*
	Mothers of euploid children n=40	23.7	5.59395	0.88448	

RU: relative units; 2 -ΔΔCT relative expression with GAPDH as housekeeping gene * significance ≤ 0.05.

Figure-1

Cyclin B1 relative expression of the studied groups



Interestingly, there were no significant differences in anthropometric measurements between mothers of SD children or mothers of euploid children. Nevertheless, statistically significant differences found in DS mothers were non-dependent on chronological age.

Discussion

Recently, maternal biological age has been associated with the risk of DS. For instance, advanced chronological age of parents, familiar chromosomopathies, and ultrasonographic changes are the conventional risk factors for DS. Globally, the maternal age-related risk factor has been considered as the most important cause of having a DS child for women of reproductive age [12]. Nonetheless, the role of cyclin B1 in the development of DS has not been investigated.

The most important risk factor in women who have a child with DS is chromosome 21 nondisjunction associated with age[13–15], but the role of the maternal genetic age has not been determined.

In this initial approach, the results showed differences in cyclin B1 expression; specifically, the mean Cyclin B1 expression level showed a higher trend in the mothers of euploid patients' group than in the mothers of DS patients' group. The low levels of this second might speed up telomere shortening in DS patients. Therefore, it would be advisable to recategorize the maternal age-related risk factor.

According to Albizua in 2016, cellular senescence and the maternal age risk factor leading to chromosome 21 nondisjunction related to biological age and not with chronological age is a new interesting target in DS risk. These results support the cyclin B/cellular senescence relationship. However, cyclin B1 expression and its role in cellular senescence is a perspective to analyze.

These findings indicate that cyclin B1 relative expression could be considered as a biomarker of the maternal age-related risk factor. Moreover, further longitudinal studies with larger samples need to be carried out in order to validate these findings[16–19]. A limitation of our study is the number of patients included. Longitudinal studies are necessary to evaluate new options for DS risk valuation.

Conclusions

The results of cyclin B1 relative expression showed significant differences between mothers of DS children and mothers of euploid children. The conventional maternal risk factor related to chronological age could be recategorized because it has been proposed that mothers of children with DS will appear "biologically older" than mothers of euploid children in a nondependent relationship with chronological age[20].

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Conflict of interests

No conflict of interests is declared.

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