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Original Article

Changes in mitochondrial DNA copy number and extracellular matrix (ECM) proteins in the uterosacral ligaments of premenopausal women with pelvic organ prolapse

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ABSTRACT

Objective: The study aimed to explore the changes in mitochondrial DNA (mtDNA) copy number, collagen, and matrix metalloproteinase (MMPs) expression with pelvic organ prolapse (POP) in the uterosacral ligaments of premenopausal women.

Materials and methods: A group of 56 premenopausal women, all younger than 52 years of age, were enrolled in this study. Uterosacral ligament (UL) biopsies were obtained from uterine specimens taken from 22 women with POP ($n = 22$, study group) and 34 myoma patients without POP ($n = 34$, control group) during abdominal or vaginal hysterectomy. Quantitative real-time polymerase chain reaction (Q-PCR) and immunohistochemistry analysis were applied in the present study.

Results: The rate of high body mass index (BMI) (> 24 kg/m^2) women was significantly higher in the POP group (81.8% vs. 35.3%, $p = 0.001$ *), and the BMI of the POP women was higher than that of the nonPOP women ($p = 0.029$ *). The mtDNA copy number ($p = 0.001$ *), collagen III alpha 1 (COL3α1) expression ($p = 0.025$ *), and MMP2 expression ($p = 0.047$ *) were significantly higher in the POP group when compared with the nonPOP group. The high BMI women had a higher mtDNA copy number ($p = 0.002$ *), COL3α1 ($p = 0.028$ *) gene expressions compared with the standard BMI women.

Conclusion: In the premenopausal state, higher BMI may be a stronger associate factor than vaginal birth for the development of POP. The higher mtDNA copy number, COL3α1, and MMP2 gene expressions are highly associated with POP in the UL of premenopausal women.

Introduction

Pelvic organ prolapse (POP) is a global health problem, affecting adult females of all ages. It decreases their quality of life considerably [1] and it is estimated that 50% of parous women have at least a minor degree of POP [2]. A woman's lifetime risk of undergoing pelvic reconstructive surgery is estimated to be ~ 11%, rendering this diagnosis one of the most common indications for surgical treatment in women [1]. Furthermore, an estimated 13–30% of women who have prolapse surgery will require a reoperation within 5 years [3]. Numerous factors, including age, ethnicity, parity, obesity, and history of hysterectomy, have been found to be associated with POP [4,5].

Despite the epidemiologic importance of this disorder, our knowledge of its pathophysiology is limited and it remains unclear how these risk factors affect the development of prolapse and which pathophysiologic mechanisms are responsible for disease manifestation [6]. This knowledge may lead to preventive measures that could eliminate or delay the onset of prolapse and improve surgical treatment [7]. The uterosacral ligaments (UL) complex in the female

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pelvis is one of the critical supportive tissues of the pelvic organs and establishes the Level 1 support of the cervix and the upper vagina [8].Studies of UL reveal that the key elements of tissue stability include the quantity, ultrastructure, and organization of extracellular matrix (ECM) proteins, such as collagens, fibronectin and elastin, as well as enzymes such as the matrixmetallo proteinases (MMPs) which participate in the degradation of collagen and other ECM proteins [7,9]. The mitochondria supply most of the cellular energy through respiration and oxidative phosphorylation. The mitochondrial DNA (mtDNA) encodes 13 essential subunits of the respiratory enzyme complexes. In our prior study, it was found that alternations in the mtDNA may play an important role in the molecular pathogenesis and formation of POP [10].

To date, many studies have evaluated the risk factors affecting the development of POP but few have evaluated how these are associated with the development of POP and the possible molecular pathophysiology in premenopausal women.

Aging and menopause are commonly accepted as promoting factors of POP [7]; however, many young premenopausal women may develop advanced POP. In this study, we only focused on premenopausal women under 52 years of age with advanced POP and tried to seek the important correlating factors and their possible pathophysiologic molecular process with regard to POP development in the premenopausal state.

Studies have revealed that being overweight is associated with the progression of POP [11] and that the prevalence of pelvic floor disorders increases with higher degrees of obesity [12]. Though POP is often a problem associated with postmenopausal women, we hypothesize that the changes in the elements of ECM and mtDNA copy number may be related to POP through an energy supplying molecular process of tissue healing remodeling in premenopausal women. In addition, we seek to establish the relationship of BMI with POP through the changes in ECM and mtDNA copy number.

Materials and methods

This study protocol was approved by the Institutional Review Board of Changhua Christian Hospital, and all patients provided informed consent before participating. We included 56 Taiwanese premenopausal female patients younger than 52 years of age with vaginal or abdominal hysterectomy performed for uterine prolapse or benign uterine myoma. 'Premenopausal' was defined as < 1 year of amenorrhea in women aged < 52 years old. Participants receiving estrogen/progesterone therapy, who had pelvic malignancies or who had undergone prior pelvic radiation, were excluded from this study.

Patients with myoma and without uterine prolapse (Stage 0) were approved by a urogynecologist and recruited as the control group; patients with prolapse (stages II–III) were the study group.

All of the 3–5 mm thick slices of biopsies from the medial ends of the bilateral UL tissue were obtained using a scalpel held between two clamps during abdominal or vaginal hysterectomy at the level of the cervical insertions to the uterus. The cervical portion of the UL tissue usually represents a reproducible, easily-accessible structure. All of the specimens were stored in a refrigerator at −80°C until DNA and RNA purification. The entire cellular DNA of the UL tissue was extracted using the PUREGENETM DNA purification kit (Gentra Systems, MN, US).

The total RNA of human UL tissue specimens was extracted with the Trizol Reagent, following the manufacturer's instructions (Invitrogen, CA, USA).

The mRNA expression of collagen I alpha 1 (COL1α1), COL3α1, matrix metalloproteinase 2 (MMP2), and MMP9 genes were analyzed via quantitative real-time polymerase chain reaction (Q-PCR). The reaction contained 10 μL 2× TaqMan Fast Universal PCR Master Mix, 1× of the primer and TaqMan FAM dye-labeled MGB probe mix for each gene, and 2 μL of 10 ngcDNA. The thermal cycler conditions consisted of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute performed by an ABI Real-Time PCR 7300 instrument (Applied Biosystems, USA). All of the samples were analyzed in duplicate and calculated by the ΔCt method.

$$\Delta Ct\ (\text{sample}) = \text{target gene Ct} - \beta\text{-actin Ct (internal control)}$$

All biopsy specimens were fixed in 10% neutral-buffered formalin for paraffin-embedded tissue sections using routine methods. Sections were cut at 4 μm thickness and mounted onto glass slides, then allowed to dry at 37°C for 12 hours. Immunohistochemistry (IHC) was performed using the Leica BOND-Max. IHC quantification uses the Image J software to measure differences in staining intensity. A total 20 ng of UL DNA was used to determine the copy numbers of mtDNA using the Applied Biosystems QPCR 7300 instrument (Tri-I Biotech, Taiwan). The primers' information was as follows: for mtDNA-ND1: Forward 5′-AACA TACCCATGGCCAACCT-3′; Reverse 5′-AGCGAAGGGTTGTAGTAGCCC-3′. For β-globin: Forward 5′-GAAGAGCCAAGGACAGGTAC-3′; Reverse 5′-CAACTTCATCCACGTTCACC-3′. The Q-PCR was modified, based on our previous report, and used to determine the UL of the mtDNA copy number [10,13].

The statistical analysis was performed using the SPSS statistical package, version 15.0 (SPSS, Chicago, IL, USA). A student *t* test was applied in the continuous variable with normal distribution (Table 1), and a Mann–Whitney U-test was applied for the comparison of the continuous variables with nonnormal distribution (Table 2). A Chi-square test was applied for the comparison between the categorical variables (Table 1). Successful log transformations into normal distribution were noted at value of mtDNA copy number, COL1α1, COL3α1, MMP2, and MMP9, using the following analysis of multiple covariate ANOVA ($p > 0.05$, One-sample Kolmogorov–Smirnov test). A multiple covariate ANOVA of a general lineal mode under controlling age, number of normal deliveries, and parity were applied in the comparison between POP patients and the normal controls (Table 2). Another multiple covariate ANOVA of the general lineal mode under controlling age and parity were applied for the comparison of higher and normal BMI (Figure 1). The statistical significance was set at a p value < 0.05.

Results

The UL of 56 women's (34 with nonprolapse, 22 with prolapse) biopsies were obtained from uterine specimens taken from 22

Table 1
Demographic characteristics of premenopausal patients with and without pelvic organ prolapse (POP).

Characteristic	nonPOP ($n = 34$)	POP ($n = 22$)	p[b]
Age (y)[a]	44.9 ± 4.1	45.7 ± 4.3	0.465
BMI (kg/m^2)[a]	23.8 ± 3.3	25.7 ± 2.7	0.029*
High BMI (%, *N*)	(35.3,12)	(81.8,18)	0.001[c]*
Parity[a]	2.6 ± 1.0	3.1 ± 1.0	0.227
NSD[a]	2.3 ± 1.2	3.1 ± 1.0	0.064
ATS (%, *N*)	(47, 16)	(23, 5)	0.066[c]

*$p \leq 0.05$.
ATS = abdominal tubal sterilization; BMI = body mass index; High BMI = BMI > 24 kg/m^2; *N* = number; nonPOP = nonpelvic organ prolapse; NSD = normal spontaneous delivery; POP = pelvic organ prolapse.
[a] Values are mean (standard deviation).
[b] Student *t* test of variance.
[c] Chi-square test.