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Expressions of homeobox, collagen and estrogen genes in women with uterine prolapse☆

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ABSTRACT

Objective: Genetic contribution is thought to be involved in the pathophysiology of pelvic organ prolapse (POP). We aimed to study the gene expression profiles of the genes HomeoboxA11 (HOXA11), HomeoboxA13 (HOXA13), Collagen Type I (COL1A), Collagen Type III (COL3A), estrogen receptor genes (ESR1 and ESR2) of round (RL) and uterosacral ligaments (USL) in postmenopausal women with uterine prolapse.

Study design: Gene expressions of 32 postmenopausal women with prolapse were analysed according to gene expressions of 8 postmenopausal women without prolapse. Quantitative real-time PCR (qRT-PCR) method was used for the detection of expression levels of the genes. Student's *t*-Test and Mann–Whitney *U* test were used for statistical analysis.

Results: In the USL specimens of all women with uterine prolapse HOXA13 and ESR1 gene expressions were decreased compared to controls (0.5 fold, $p = 0.04$ and 0.82 fold, $p = 0.04$, respectively). In the RL specimens, ESR2 gene expression was decreased 0.7 fold in women with prolapse when compared to controls ($p = 0.04$).

In the USL specimens of women with advanced stages of prolapse (stage ≥ 3), HOXA13 and COL3A gene expressions were decreased compared to controls (0.44 fold, $p = 0.043$ and 0.39 fold, $p = 0.045$, respectively). In the RL specimens, ESR2 gene expression was decreased 0.65 fold in women with prolapse when compared to controls ($p = 0.052$).

Conclusion: The significant decrease in the expression of the genes HOXA13, COL3A in the USL and ESR2 in the RL especially in advanced stages of prolapse, implicate that these gene expressions may play a role in the development of uterine prolapse.

Introduction

Pelvic organ prolapse (POP) is a common public health problem affecting approximately one half of women after 50 years of age, with significant consequences on health, quality of life and health costs [1–4]. Defining the aetiology and risk factors of this highly prevalent condition is of paramount importance in determining individuals at risk in order to provide preventive strategies, or in planning the most appropriate management option for affected individuals.

The aetiology of POP is multifactorial; vaginal birth, parity, aging, menopause, obesity, chronic constipation and chronic obstructive lung disease are considered as risk factors for POP [4]. Still, neither of these risk factors alone nor in combination are enough to explain the genesis of POP in all women, probably because of individual differences in genetic variations. Indeed, genetic predisposition is considered an important risk factor in the aetiology of POP, as suggested by molecular and family studies [5]. Many genes have been studied in this context, such as; genes that take part in the development of lower reproductive tract, genes that code the major components of extracellular matrix (ECM) and genes that code estrogen receptors (ER) [6–18].

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Homeobox (HOX) genes are evolutionary highly conserved regulatory genes that encode transcription factors which are needed in the embryonic development [6]. Mutations or altered gene expression in the defined 39 HOX genes are reported to be causing human disorders. All these genes are classified in 4 clusters namely A, B, C and D; where HOXA genes especially control the reproductive system [7,8].

HOXA11 has been reported to be responsible for the development of uterosacral ligaments (USL), lower uterine segment and cervix, as well as synthesis of collagen type III and matrix metalloproteinase 2 (MMP2) [9,10]. Similarly, HOXA13 gene has been reported to regulate the ECM constituents [11,12].

In an experimental study, it has been shown that the knockdown of HOXA11 gene in vivo in the USL of mice and in an in vitro model performed by fibroblasts, confirmed that HOXA11 gene has a key role in the homeostasis of connective tissue support [9]. However, in studies evaluating the HOXA11 gene expression in women with POP, some reported a decrease, while others reported no difference in HOXA11 mRNA expression [10,13].

The homeostasis of ECM, especially the synthesis and destruction of collagen and elastin is important in the maintenance of pelvic floor integrity; POP has been shown to be associated with changes in collagen metabolism, quantity and composition of different collagen subtypes [14–16]. More than 20 subtypes of collagen have been identified. Collagen type I is non-elastic and confers great resistance to tensile forces and collagen type III has elastic properties and is widely found in more flexible tissues. Changes in collagen type III and I ratios may result in connective tissue disorders [16].

Although the prevalence of POP increases in the post-menopausal period and the hypo estrogenic state may contribute to its development, the precise role of estrogen in the pathogenesis of POP is still unclear and there are conflicting results on this subject in the literature [19–21]. Estrogen activity is dependent not only on serum estrogen level, but also on the expression of ER, thus the effect of estrogen in connective tissue as a target organ depends not only on the exposing levels of estrogen but also intracellular system of estrogen signalling expression and their co-regulators [17].

The USL are the main supportive structures of the cervix and proximal vagina, which is described as level 1 support [22]. Gene expressions in POP have been studied in USL and also in other tissues, such as the RL and vaginal wall, as they are all continuations and thus, thought to be reflections of the endopelvic fascia. However, it has also been shown that these tissues differ in structure and expression of markers of collagen metabolism, leading to the idea that their structure and, gene expressions may be affected by prolapse [23–25].

Thus, in this study, it was aimed to evaluate the expression profiles of the genes HOXA11, HOXA13, COL1A, COL3A, ESR1 and ESR2 in the supporting ligaments of the uterus in postmenopausal women with uterine prolapse.

Materials and methods

Among postmenopausal women with POPQ stage ≥2 uterine prolapse [26] admitted to Ankara University School of Medicine, Department of Obstetrics and Gynaecology hospital for vaginal hysterectomy, those without cancer, connective tissue disorders and those without a history of hormone replacement therapy comprised the study group (n = 32).

Ethical approval was taken from the Ethical Committee of the University (Institutional Board Registration number: (13-426-12). All patients provided informed consent before participating the study. During the hysterectomies, round (RL) and uterosacral ligament (USL) specimens were obtained in standard steps for the analysis of gene expression levels.

Standard steps of specimen collection

During hysterectomy, the round (RL) and uterosacral ligaments (USL) were grasped 2 cm proximal to the uterus with a toothless forceps; 1 cm pieces of ligaments were cut with a scalpel at their attachment points to the uterus carefully, without traumatising the tissue. The ligaments were immediately immersed in Trizol solution and were stored at −80 °C until RNA purification.

Gene expression analysis

After the collection of all specimens, quantitative real-time PCR (qRT-PCR) method was used for the detection of HOXA11, HOXA13, COL1A, COL3A, ESR1 and ESR2 gene expression levels, at the Department of Medical Biology of Ankara University, School of Medicine.

Total RNA was extracted from tissue samples by using the Trizol (Sigma) reagent according to the manufacturer's protocols. cDNA was generated from RNA by reverse transcriptase (Transcriptor High Fidelity cDNA Synthesis Kit; Roche). qRT-PCR was performed using SYBR Green PCR Master Mix (Roche) on LC480 instrument. mRNA was measured relative to HPRT gene (hypoxanthine phosphoribosyltransferase) as an endogenous control. The primer sequences used for the genes are given in Table1.

The threshold cycle (Ct) number was determined and was used in the comparative Ct method. The fold changes of the gene expression levels of the study group were calculated according to the gene expression levels of the specimens collected with the aforementioned steps from postmenopausal women without uterine prolapse (n = 8) who underwent abdominal hysterectomy in the same department. The calculation of fold change in the gene expression levels was performed with the 2-ΔΔCT method [27–29]. Gene expression levels had a wide variability, thus, we computed logarithmic values.

The gene expression levels of women with advanced uterine prolapse (stage ≥3, n = 15) were further analysed in order to see if there is any remarkable change in the expression of genes with increasing severity of prolapse.

Statistical analysis was performed using SPSS Version 11.5; Student's *t*-Test, Mann–Whitney *U* Test and One Sample *t*-Tests were used as appropriate. $P < 0.05$ was considered statistically significant.

Results

Parity, family history of POP, chronic diseases, smoking, steroid use and history of pelvic surgeries were similar in women with uterine prolapse and controls. Women with uterine prolapse were older (Mean 65,25 ± 8,42 years vs 57,00 ± 6,59 years, p = 0,014), their menopause duration was longer (16,80 ± 8,40 years vs

Table 1
Primer sequences of the genes.

HOXA11 F: CAGTCTCGTCCAATTTCTATAGCA
HOXA11 R: TCATTCTCCTGTTCTGAAACCA
HOXA13 F: GAGTTCGCCTTCTACCACC
HOXA13 R: ATATCCGCCTCCGTTTGTC
COL1A1 F: TGGCTATGATGAGAAATCAACC
COL1A1 R: CCCATCATCTCCATTCTTTCC
COL3A1 F: CTGTGAATCATGCCCTACTGGTC
COL3A1 R: AAGCCTCTGTGTCCTTTCATACC
ESR1 F: CTGATGATTGGTCTCGTCTG
ESR1 R: AAAGTGTCTGTGATCTTGTCC
ESR2 F: GGATGGAGGTGTTAATGATGG
ESR2 R: GAGGGTACATACTGGAATTGAG

F: Forward, R: Reverse.
HOXA11: HomeoboxA11; HOXA13: HomeoboxA13; COL1A; Collagen Type I; COL3A; Collagen Type III, ESR1: Estrogen receptor 1; ESR2: Estrogen receptor 2.

10,00 ± 6,67 years, p = 0.025), and their maximum birth weight delivered was higher (3658,69 ± 351,50 g vs 3312,50 ± 513,91 g, p = 0.042). BMI was higher in women without prolapse (32,13 ± 3,70 kg/m^2 vs 29,37 ± 3,01 kg/m^2, p = 0.035) (Table 2).

Gene expression fold changes compared to control are shown in Table 3. In the USL specimens of all women with prolapse; HOXA13 and ESR1 gene expressions were decreased compared to controls (0.5 fold, p = 0.04 and 0.82 fold, p = 0.04, respectively), but, HOXA11, COL1A, COL3A and ESR2 gene expressions were found similar (p > 0.17). In the RL specimens, ESR2 gene expression was decreased 0.7 fold in women with prolapse when compared to controls (p = 0.04); expression levels of HOXA11, HOXA13, COL1A, COL3A and ESR1 were similar in women with prolapse and controls (p > 0.098).

In the USL specimens of women with advanced stages of prolapse (stage ≥3), HOXA13 and COL3A gene expressions were decreased compared to controls (0.44 fold, p = 0.043 and 0.39 fold, p = 0.045, respectively). HOXA11, COL1A, ESR1 and ESR2 gene expressions were found similar (p > 0.113). In the RL specimens, ESR2 gene expression was decreased 0.65 fold in women with prolapse when compared to controls (p=0.052); expression levels HOXA11, HOXA13, COL1A, COL3A and ESR1 were similar in women with prolapse and controls (p > 0.064).

Table 3
Gene expression changes in all women with prolapse and women with advanced stages of prolapse (stage ≥3) compared to the control group.

Gene	Gene expression fold change compared to control							
	All prolapse stages				Advanced prolapse stages			
	USL	p	RL	p	USL	p	RL	p
HOXA11	1.15	0.272	0.81	0.553	0.50	0.113	0.63	0.547
HOXA13	0.5	**0.041**	0.54	0.114	0.44	**0.043**	0.29	0.064
COL1A	0.89	0.660	0.70	0.410	1.90	0.779	1.17	0.331
COL3A	0.69	0.166	0.84	0.098	0.39	**0.045**	0.84	0.208
ESR1	0.82	**0.04**	0.89	0.135	0.93	0.170	0.81	0.369
ESR2	0.81	0.564	0.70	**0.043**	0.68	0.159	0.65	0.052

P < 0.05 statistically significant.
USL: Uterosacral ligaments; RL: Round ligaments.
HOXA11: HomeoboxA11; HOXA13: HomeoboxA13; COL1A; Collagen Type I; COL3A; Collagen Type III, ESR1: Estrogen receptor 1; ESR2: Estrogen receptor 2.

Comment

In this study, statistically significant changes in gene expressions in the USL and RL of women with prolapse were found. For all women with prolapse, HOXA13 and ESR1 gene expressions in the USL, and ESR2 gene expression in the RL were found significantly decreased compared to controls. With advanced stages of prolapse, the decrease in HOXA13 expression remained significant; additionally, a decrease in COL3A gene expression in the USL and ESR2 gene expression in the RL became significant, compared to controls.

HOXA13 expression was found to be lower in the USL of women with POP compared to women with normal pelvic support in our study. This is in accordance with the literature; it has been reported that HOXA13 gene expression in premenopausal women with prolapse was 14 folds lower than controls (p < 0.005) [12]. In our study, the decrease in the level of expression was less (0.5 folds), and the study group was comprised of postmenopausal women. It has been shown that the decrease in HOXA13 gene expression was similar in premenopausal and postmenopausal POP patients suggesting that the gene expression was not affected by menopause [12]. In the same study, inducing a hypo estrogenic state in premenopausal women with leuprolide acetate also did not affect HOXA13 gene expression, which is a finding that has been proposed to indicate that estrogen and HOXA13 work through separate pathways in the extracellular matrix metabolism of the vagina [12].

Studies have reported conflicting results regarding the level of HOXA11 gene expression in women with POP [10,13,18]. Some studies reported a decrease in HOXA11 gene expression in POP patients [13,18], while others reported no difference in HOXA11 mRNA expression [10]. In mice, it has been shown that the deficient HOXA11 signalling may contribute to alterations in the biochemical strength of the USL, leading to pelvic organ prolapse [3]. However, it is unknown how HOXA11 transcripts are regulated in the USL. It has been suggested that, the effect of other factors found in chromatin structure such as micro RNAs (mi RNAs), may play role in the regulation of gene expression [30]. In addition, MMPs that take place in the destruction of matrix proteins have also been suggested to be important and correlated with tissue strength of POP patients by affecting HOXA11 gene expression and collagen metabolism [9,31].

In our study, we did not observe a significant change in the expression of HOXA11 gene in the USL and RL of women with POP compared with the control group. However, the significant decrease in the gene expression profile of COL3A in the USL in women with advanced prolapse may be correlated with this finding, as HOXA11 is known to be coding and regulating for COL3A [9,13].

Decreased COL1A and COL3A gene expression levels in USL of POP patients have been reported [13]. On the other hand, Gabriel et al have shown higher collagen III expression, but no change in collagen I in USL of postmenopausal women with POP [32]. We

Table 2
Demographic characteristics of women with uterine prolapse and controls.

	Control N = 8	Uterine prolapse N = 32	p
Age years, mean ± SD	57.00 ± 6.59	65.25 ± 8.42	p = 0.014
BMI kg/m^2, mean ± SD	32.13 ± 3.70	29.37 ± 3.01	p = 0.035
Menopause duration years, mean ± SD	10.00 ± 6.67	16.80 ± 8.40	p = 0.025
Parity n, median (min–max)	4.00 ± 3.20	3.62 ± 1.87	p = 0.753
Birthweight g, mean ± SD	3312.50 ± 513.91	3658.69 ± 351.50	p = 0.042
Family history of POP n(%)	%37.5	%43.8	p = 0.537
Chronic constipation n(%)	%0	%12.5	p = 0.393
COLD n(%)	%25	%12.5	p = 0.344
Steroid n(%)	%0	%6.2	p = 0.636
Autoimmune disease n(%)	%0	%6.2	p = 0.636
Smoking n(%)	%12.5	%3.1	p = 0.364
Pelvic surgery n(%)	%25	%12.5	p = 0.344

POP: Pelvic organ prolapse, BMI: Body mass index, COLD: Chronic obstructive lung disease.
P < 0.05 is considered as statistically significant.

observed a significant decrease for COL3A gene expression only in the USL of women with *advanced POP*, and we could not find any change in the gene expression level of COL1A. The discrepancies may be due to not only the complex mechanisms involved in collagen synthesis and breakdown, but also to the molecular structure of collagen as reflected by the differences in genetic variants of genes [14]. Single nucleotide polymorphism studies have shown different variants of collagen genes in different populations [5,14].

Studies investigating the role of ER in the development of prolapse in USL have also reported conflicting results. In our study, ESR1 gene expression in USL was reduced significantly in POP patients. Shi et al., reported reduced expression of ER-α in the USL, which is the protein coded by the estrogen gene, and explained this decrease by the elevated miR-221/222 expression levels. They suggested that it may be associated and responsible for the reduced ER-α expression in the cervical portion of USL of POP patients and it may serve as a potential therapeutic target for POP [33].

A group of investigators, using western blot technique, showed that expression level of ER-β 1 was lower in the USL of POP patients when compared to the control group [34]. They also compared the expression levels of the related genes between POP stages 3 and 2; stage 2 group showed higher expression than stage 3 so they suggested that, ER-β1 levels could also predict the severity of POP [34]. In our study, ESR2 gene expression in the USL was similar with controls; however, the gene expression level in RL was decreased significantly in prolapse patients and remained significant in patients with stage $\geq$3 prolapse.

In conclusion, our study revealed significant decrease in the expression of the genes HOXA13, COL3A in the USL and ESR2 in the RL especially in advanced stages of prolapse, implicating that these gene expressions may play a role in the development of uterine prolapse.

Conflicts of interest

None.

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