ORIGINAL RESEARCH

Association of HOXA13 Gene Expression among Premenopausal Women with the Severity of Pelvic Organ Prolapse: A Cross-sectional Study

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Abstract

Aim: To determine the association of HOXA13 gene expression in vaginal wall tissue with the severity of pelvic organ prolapse (POP) among premenopausal women.

Materials and methods: A cross-sectional study was conducted on a total of 60 premenopausal women. Subjects with ≥stage II POP were enrolled as cases, while those with benign gynecological conditions other than prolapse were taken as controls. Vaginal tissues were obtained during surgical procedures and HOXA13 gene analysis was done using real-time polymerase chain reaction. Spearman rank correlation coefficient was used for the correlation of true fold change of HOXA13 gene with other parameters.

Results: Overall, HOXA13 gene was observed 1.21-fold downregulated in women with POP ($p = 0.38$). The gene was diminished in higher stages (stage III and stage IV) of POP ($p = 0.007$). It was found downregulated in most (84.21%) of the females above 40 years ($p = 0.01$).

Conclusion: Downregulation of HOXA13 gene was seen in the majority of the women with POP, though not statistically significant. The gene expression was significantly diminished in women with advanced stages of prolapse (stage III and stage IV) as well as in women with age above 40 years.

Clinical significance: Downregulation of HOXA13 gene can be one of the etiological factors of POP. Hence, preventive strategies may be developed using its gene expression analysis in future.

Keywords: Pelvic organ prolapse, Premenopausal women, Severity.

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Introduction

Pelvic organ prolapse is a bulge or protrusion of pelvic organs and their associated vaginal segments into or through the vagina.[1] POP are usually clinically silent; therefore, its exact prevalence is not known. More than half of the females with age more than 50 years suffer from symptoms of prolapse like sensation of pelvic pressure or vaginal "heaviness" and recurrent irritative bladder symptoms or defecatory difficulties.[2]

Pelvic organ prolapse being a multifactorial disease is associated with race, age, parity, body mass index, hormonal status, smoking, socioeconomic status, and obstetric factors like parity, vacuum, or forceps delivery, etc.[3] However, these environmental factors do not give complete explanation of etiology as some women with multiple risk factors never develop prolapse while women with no risk factors develop prolapse.[4] So, there is some role of biochemical and molecular mechanisms which need to be more clearly identified.

Female pelvic organs are structurally supported by pelvic floor muscles, cardinal and uterosacral ligaments, and the endopelvic fascia.[5] Collagen (part of endopelvic fascia) may undergo some changes due to genetic variations leading to weakening of fascia as well as decrease in vaginal resistance.[6] The disturbance in complex interaction between levator ani, vagina, and its connective tissues comprising extracellular matrix results in loss of vaginal support and thus POP.[7]

Homeobox genes, which are evolutionarily conserved genes, encode transcription factors that regulate mammalian embryonic growth and development of urogenital tract. HOXA cluster genes mediate segmental differentiation of paramesonephric duct into morphologically distinct organs of female reproductive tract.[7] After birth, a spatial HOX axis is established. HOXA9 is expressed in fallopian tube, HOXA10 in uterus, HOXA11 in uterus and cervix, and HOXA13 in upper vagina. Regulation of extracellular matrix is done by these HOX genes.[8] HOXA13 gene is involved in upregulation of various collagen types that provide tensile strength to the constituents of vagina as well as basement membrane. It also upregulates fibulin, fibrillin 1, laminin 2 and 3, and osteonectin.[9] Mutation of these constituents has shown to be associated with POP in women.[10,11]

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Some studies have been conducted regarding HOXA11 gene and its association with POP.[12–14] Still there is paucity of information regarding role of HOXA13 gene which is a regulatory gene in upper vagina with the severity of POP. Few studies have shown association of HOXA13 gene with POP.[15,16] Still there is need to have clarity over its contribution to POP especially in young women where the etiology is yet to be established.

With this background, the present study was planned to determine expression of HOXA13 gene in upper vagina in premenopausal women presenting with POP and then associate it with the severity of POP.

Materials and Methods

A cross-sectional study was conducted on a total of 60 premenopausal women. The primary objective was to determine the association of HOXA13 gene expression in vaginal wall tissue with the severity of POP among premenopausal women. The secondary objectives were firstly to compare its expression in premenopausal women with POP with that of age-matched premenopausal women without POP; secondly, to find the pattern of expression with respect to age of premenopausal women.

An ethical clearance from institutional ethical committee for human research as well as written consent from the subjects was taken. A detailed history was obtained, and examination was done in each subject recruited. Women with any malignancy, history of steroid intake or pelvic surgeries, collagen disorders like Ehlers-Danlos syndrome and Marfan syndrome and with BMI ≥30 kg/m^2 were excluded out. Quantification of prolapse was done as per the POP quantification (POPQ) system and stage of prolapse was assigned accordingly in women presenting with findings suggestive of POP. Women with ≥stage II planned for any surgical procedure for POP were grouped into cases. Those who presented with other benign gynecological conditions like leiomyoma and decided for hysterectomy (any route) were taken as controls.

HOXA13 gene expression analysis was done by obtaining around 0.5 x 0.5 cm tissue sample from upper part and anterior vaginal wall of enrolled patients during the surgical procedures. They were stored at −20°C after fixing in TRIzol reagent for further expression studies. Total RNA isolation was done by TRIzol method followed by complementary DNA synthesis of microRNA using commercially available kit (Fermentas, Thermoscientific). Finally, quantitative expression of mRNA was done using real-time quantitative PCR (qRT PCR) with specific primer and fold change of HOXA13 gene was calculated with respect to 30 women without POP.

The data were entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in numbers and percentages (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then nonparametric test was used. Statistical tests were applied. Quantitative variables were compared using independent *t* test/Mann-Whitney test (when the data sets were not normally distributed) between the two groups and ANOVA/Kruskal-Wallis test between more than two groups. Qualitative variables were compared using Chi-square test/Fisher's exact test. Spearman rank correlation coefficient was used to assess the correlation of true fold change of HOXA13 gene with other parameters. A *p* value of <0.05 was considered statistically significant.

Results

During the study period, 60 women (30 cases having POP and 30 controls without POP) underwent various surgical procedures during which 0.5 × 0.5 cm vaginal tissue was obtained from both the groups. HOXA13 gene expression analysis was done and compared among the groups.

In the present study, the mean age (years) of the subjects in case group was 42.7 ± 4.66 and in control group was 41.9 ± 5.39; not statistically significant (0.60). The mean BMI (kg/m^2) of cases was 24.26 ± 2.2 and of controls was 24.85 ± 2.44, not statistically significant (0.44).

The common complaints among the cases were something coming out of vagina (100%), heaviness in perineum (73.3%), backache (70%), and difficulty in reducing the prolapse (66.6%). The main presenting symptom among control group was bleeding per vaginum. Nearly 16.67% gave history of spotting per vaginum. Pain in the lower abdomen was present in 36.67% while discharge per vaginum was seen in 10% of controls.

Multiparous women were 93.3% in case group and 83.4% in control group. About 6.7% of women with POP and 3.3% of women without POP were grand multipara. No female was primipara in case group. It was not statistically significant ($p = 0.105$). The mean parity in case group was 3.80 ± 1.35 and in control group was 2.70 ± 1.37 which was found statistically significant ($p = 0.003$).

Majority (66.67%) in each group had normal deliveries, not statistically significant ($p = 0.251$). The average age at the time of first childbirth was 24.28 years, not statistically significant ($p = 0.34$). Nearly 40% of the deliveries in women with POP and 26.67% in women without POP were home deliveries. Of these, almost 30% of the deliveries were assisted by untrained dais. Both were statistically not significant ($p \geq 0.05$). None of the females gave history of instrumentation. History of birth weight could not be elicited due to long time gap.

Majority of the cases (60%) were having stage III-C prolapse followed by stage IV prolapse which was seen in 26.67% of the subjects. Approximately 10% of the cases were having stage II-C prolapse while only 3.33% of the subjects were observed with stage III-Bp prolapse.

On taking 30 subjects with POP and 30 subjects with normal pelvic support, 1.21-fold change downregulation of HOXA13 gene was seen in subjects having POP. It was not statistically significant ($p = 0.38$).

On taking the mean of HOXA13 gene delta CT values for both controls as well as cases and evaluating the expression of HOXA13 gene in each patient against it, it was found that the gene was downregulated in 19 patients. Also, the gene was downregulated in majority of the cases on the individual level.

Association of severity of the stage of prolapse was done with the fold change of HOXA13 gene, and it was observed that downregulation of HOXA13 gene was seen in stage III and stage IV prolapse, i.e., with advanced stages of prolapse. The association was found statistically significant ($p = 0.007$) as shown in Table 1.

Table 1: Association of severity of POP with fold change of HOXA13 gene

Stage	*Downregulation (n = 19) n (%)*	*Upregulation (n = 11) n (%)*	*Total n (%)*	*p value*
II	0 (0%)	3 (27.27%)	3 (10%)	
III	11 (57.89%)	8 (72.73%)	19 (63.33%)	0.007
IV	8 (42.11%)	0 (0%)	8 (26.67%)	

Table 2: Association of age (years) with fold change of HOXA13 gene

Age (years)	*Downregulation (n = 19) n (%)*	*Upregulation (n = 11) n (%)*	*Total n (%)*	*p value*
≤40	3 (15.79%)	7 (63.64%)	10 (33.33%)	0.015
>40	16 (84.21%)	4 (36.36%)	20 (66.6%)	

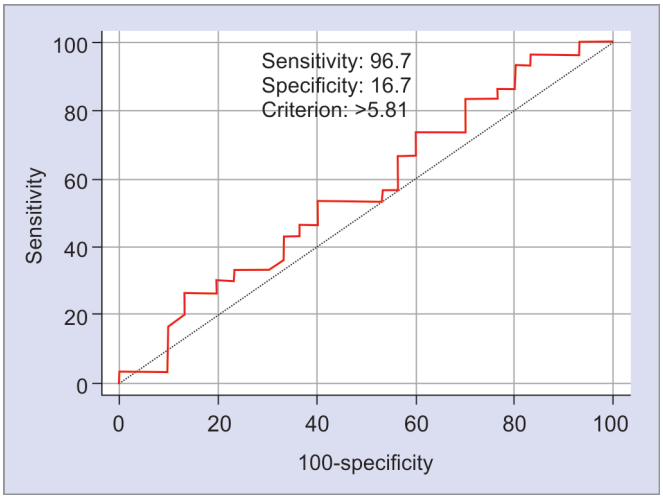

Fig. 1: Receiver operating characteristic curve to find out the cut-off point of HOXA13 gene expression (delta CT) for predicting prolapse

Association of age of women with POP was done with fold change of HOXA13 gene and it was observed to be downregulated in most (84.21%) of the females above 40 years. It was found statistically significant ($p = 0.01$) as depicted in Table 2.

The sensitivity, specificity, and predictive values were calculated using HOXA13 delta CT values to find out the cut-off value in order to predict prolapse. The sensitivity and specificity of HOXA13 gene analysis for predicting prolapse were 96.67 and 16.67%, respectively. The positive predictive value and negative predictive value of HOXA13 gene analysis for predicting prolapse were 53.7 and 83.3%, respectively. The cut-off value for predicting prolapse in terms of HOXA13 delta CT was >5.81 as shown in Figure 1.

Discussion

Pelvic organ prolapse among young women is relatively less as compared to the elderly population. Many studies have been conducted worldwide regarding various risk factors responsible for POP in postmenopausal women. However, there is still lack of information over POP and its related etiology in young females.

In the present study, women having BMI ≥30 kg/m^2 were excluded from the study as obesity directly affects the symptoms and acts as an independent risk factor for POP.[17] The mean BMI of premenopausal women without prolapse was 24.55 ± 2.32 kg/m^2 and with prolapse was 24.26 ± 2.2 kg/m^2. It was not found statistically significant ($p = 0.446$) in the present study. Connell et al. obtained the mean BMI of premenopausal controls as 28.1 ± 6.6 kg/m^2 and in cases as 27.6 ± 6.8 kg/m^2 which was not statistically significant ($p = 0.90$).[17] The study done by Dokmeci et al. depicted that the mean BMI was 32.13 ± 3.70 kg/m^2 in postmenopausal control group and 29.37 ± 3.01 kg/m^2 in postmenopausal case group which was statistically significant ($p = 0.035$).[16] There are high chances of pelvic floor dysfunction in individuals having obesity and its related comorbidities.[18,19] It has been observed through various studies that there is a 2.5-fold increased risk of POP in obese women as compared to women with normal BMI. Also, there is 3% high risk of development of POP with each unit of increasing BMI.[20]

The mean parity in the present study of control group was 2.7 ± 1.37, and of case group, it was 3.8 ± 1.35. It was obtained statistically significant ($p = 0.003$) suggesting multiparity as one of the contributing factors for POP. The study conducted by Connell et al. depicted mean parity in premenopausal females with POP as 2.38 ± 1.0 and without POP as 1.7 ± 1.6. It was found not statistically significant ($p = 0.34$).[15] According to the study done by Dokmeci et al., median parity of postmenopausal controls was 4 and for cases was 3 which was not statistically significant ($p = 0.75$).[16] Multiparity may be one of the strongest risk factors for POP. Women with one child show fourfold increased likelihood of POP while women with two children have 8.4 times more risk of developing POP.[17]

In the present study, HOXA13 gene expression was elicited in premenopausal women with POP and was observed to be 1.21 times downregulated as compared to women without POP. Among women with POP, downregulation was present in 63.3% while the remaining demonstrated upregulation. However, it was not statistically significant ($p = 0.38$). Also using HOXA13 delta CT values, sensitivity, specificity, negative, and positive predictive values for using HOXA13 gene expression in predicting prolapse were calculated in the present study. Sensitivity found was 96.67%, specificity was 16.67%, positive predictive value was 53.7%, and negative predictive value was 83.3%. The study performed by Connell et al. demonstrated significant (<0.001) downregulation of HOXA13 gene which was 14-fold in premenopausal women with POP and also revealed that it was not affected by menopause or hormone (observed by giving leuprolide acetate).[15] The study by Dokmeci et al. depicted expression of HOXA13 in uterosacral ligaments of postmenopausal women with POP and found 0.5 times downregulated as compared to controls significantly ($p = 0.04$). The study also showed downregulation of other genes in uterosacral ligaments such as COL1A, COL3A, ESR1, and ESR2 gene out of which ESR1 gene downregulation was statistically significant ($p = 0.04$).[16]

Also, in the present study, the association of HOXA13 gene with the severity of stage of prolapse was obtained statistically significant ($p = 0.007$). In advanced stages of prolapse (stage III and stage IV), mostly downregulation of HOXA13 gene was observed. However, studies relating such association were not found. In the present study, the association of fold change of HOXA13 gene expression with the age of subjects with POP was statistically significant ($p = 0.01$). It was found downregulated in most of the subjects with age above 40 years. With each decade of life, there is 10% increased risk of POP according to the POSST study.[21] According to the study conducted by Reay Jones et al., there was a significant decrease in uterosacral ligament resilience (UsR) which was measured by tensiometry with menopause ($p = 0.009$) and older age ($p = 0.005$), in patients with and without POP.[22] Thus, there is decrease in pelvic connective tissue elasticity in case of weakened pelvic floor muscles with increasing age and menopause.

The present study has few limitations. The sample size was small. Also, only premenopausal women were included in the study. Hence, the effect of menopause over HOXA13 gene expression could not be elucidated.

Conclusion

Downregulation of HOXA13 gene expression has been observed in premenopausal women with POP, though not statistically

significant. However, HOXA13 gene downregulation is significantly associated with the increasing severity of POP. It has also been seen significantly associated with the increasing age of women. Women with POP were mostly multipara which also was significantly obtained. Thus, the present study depicts a crucial role of HOXA13 gene (embryologically related to pelvic structures) in the etiology of POP. However, larger longitudinal studies may be done in more number of premenopausal women as well as in other pelvic supports of uterus to prevail clearer view about its role in the etiology of POP for development of better strategies for its prevention and treatment.

Clinical Significance

HOXA13 gene seems to play a role in maintaining the strength of vagina. More the gene is downregulated, higher could be the severity of POP. Therefore, better strategies for screening can be developed in future for prevention of prolapse such as taking biopsies from vagina and analyzing the gene expression.

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