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BIOCHEMICAL EVALUATION ON THE SUPPORTING STRUCTURE OF PELVIC ORGANS IN PRE AND POST MENOPAUSAL WOMEN

Hypothesis / aims of study

The uterosacral ligament provides support to the uterus, cervix and the apical portion of the vagina. There is evidence to suggest that the menopausal status may predispose women to the development of pelvic organ prolapse (POP) due to possible changes in the biochemical components in the pelvic supporting system. While childbirth and chronic stress related to obesity have been cited to predispose to pelvic organ prolapse (POP), the integrity of supporting tissues like the utero-sacral ligaments are clearly related to alterations in the biochemical components in the extra-cellular matrix (ECM) distinctly seen following oestrogen deprivation after menopause[(1-3)]. Although there is ample data in research conducted amongst Western women, there is scant data on the precise alterations that occur in the extracellular matrix (ECM) proteins of these structures in the perimenopausal Malaysian population.

The aims of the study are to determine ECM proteins by semi-quantitative evaluation in three groups of women drawn from three hospitals in Malaysia and to determine if there are differences in specific proteins viz. collagen-1, collagen-3, ratio of collagen1:3, matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-2 (MMP-2), elastin and tenascin in three groups of women namely pre-menopausal (Group-1), post-menopausal without POP (Group-2) and post-menopausal with POP (Group-3).

Study design, materials and methods

Thirty women who met the inclusion criteria and consented for the study were recruited. Specified site of the utero-sacral ligaments were sampled after hysterectomy for indicated benign disease. Both abdominal and vaginal approaches were admitted for this study. The samples were fixed in formalin following standard procedures. Immunohistochemical staining was performed on paraffin-embedded sections of the biopsy samples. The women were categorized accordingly into premenopausal (group 1), postmenopausal (group 2) and postmenopausal with POP (group 3). Their age, duration of menopause, Body Mass Index (BMI), parity and vaginal deliveries were documented. The mean percentage area stained for collagen I, collagen III, MMP-1, MMP-2, elastin and tenascin was quantified by image J software analysis for all 3 groups. Kruskal- Wallis and Mann Whitney test were used in all comparisons.

Results

Table 1: Clinical Profiles of Premenopausal and Postmenopausal Women with and without Pelvic Organ Prolapse

Groups ***Clinical Parameter***	***Group 1*** ***(PR)***	***Group 2*** ***(PO)***	***Group 3*** ***(PO/P)***	***P-Value***
Age (Years) (Mean ± SEM)	45.6 ± 1.4 **PO **PO/P	60.5 ± 4.1 **PR	68.3 ± 4.1 **PR	0.0001 (Significant)
Duration of Menopause (Years) (Mean ± SEM)	-	12 ± 2.9	17.8 ± 1.0	0.3284 (Insignificant)
Body Mass Index (B.M.I) (Mean ± SEM)	25.1 ± 1.5	26.9 ± 1.3	25.3 ± 1.2	0.7300 (Insignificant)
Number of Parity (Mean ± SEM)	1.8 ± 0.5	3.5 ± 0.8	2.0 ± 0.9	0.3545 (Insignificant)
No. of Spontaneous Vaginal Delivery (Mean ± SEM)	1.6 ± 0.5	3.2 ± 1.0	2.0 ± 0.9	0.5492 (Insignificant)

The table shows the clinical profile of the premenopausal and postmenopausal women with and without POP expressed in mean ± standard error of mean. P-value <0.05) is significant

Table 2: Mean Percentage Area of ECM Proteins

Biochemical evaluation of ECM	***Group 1*** ***(PR)***	***Group 2*** ***(PO)***	***Group 3*** ***(PO/P)***	***P-Value***
Collagen 1	63.8 ± 2.2	48.6 ± 5.8	40.7 ± 2.6	<0.05

Collagen 3	41.1 ± 1.3	46.3 ± 3.3	77.8 ± 0.8	<0.01
Ratio of Collagen 1:3	1.6 ± 0.1	1.1 ± 0.1	0.5 ± 0.04	<0.05
MMP- 1	20.7 ± 1.3	40.0 ± 2.5	58.4 ± 2.7	<0.05
MMP- 2	25.9 ± 1.1	25.9 ± 0.92	32.0 ± 2.2	insignificant
Elastin	40.8 ± 2.2	27.9 ± 2.4	19.8 ± 1.9	<0.05
Tenascin	10.7 ± 0.8	22.5 ± 2.1	36.3 ± 3.2	<0.01

Interpretation of Results

The mean age of group 3 was the highest (68.3 +/-4.1) compared to the other groups (Gp 1: 45.6+/- 1.4, Gp 2: 60.5+/-4.1) but was not significantly different from group 2. The duration of menopause was longer in group 3(17.8+/-1.0 years) compared to group 2 (12 +/- 2.9 years) though not statistically significant. Other clinical parameters showed no significant different in all groups. The uterosacral ligament from postmenopausal women (group 2) was characterized by a lower expression of collagen I, including ratio of collagen I: collagen III and elastin, but higher expression of MMP-1 and tenascin as compared to premenopausal women (group 1). Further, it was observed that the changes in ECM expression appeared to be exaggerated in postmenopausal women with POP (group 3) as compared to group 2. However, the difference in these 2 groups was not statistically significant

Concluding message

The study confirms the pattern of ECM in the utero-sacral ligaments drawn from Malaysian women to be similar to women in other countries. A significant difference in the expression of ECM proteins was apparent when premenopausal subjects were compared to postmenopausal subjects (p =0.05). Clearly there is a compromise of utero-sacral ligament tensile strength as demonstrated by the differences of ECM proteins. Although exaggerated difference in the quality of ECM proteins were demonstrated the sample size limited showing statistical significance. The duration of menopause impacted on the increased possibility of the occurrence of POP.

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Is this a Randomised Controlled Trial (RCT)?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**