#26148 Analysis of HOXA11 gene expression and histomorphology in the uterosacral ligament and vaginal wall in women with pelvic organ prolapse

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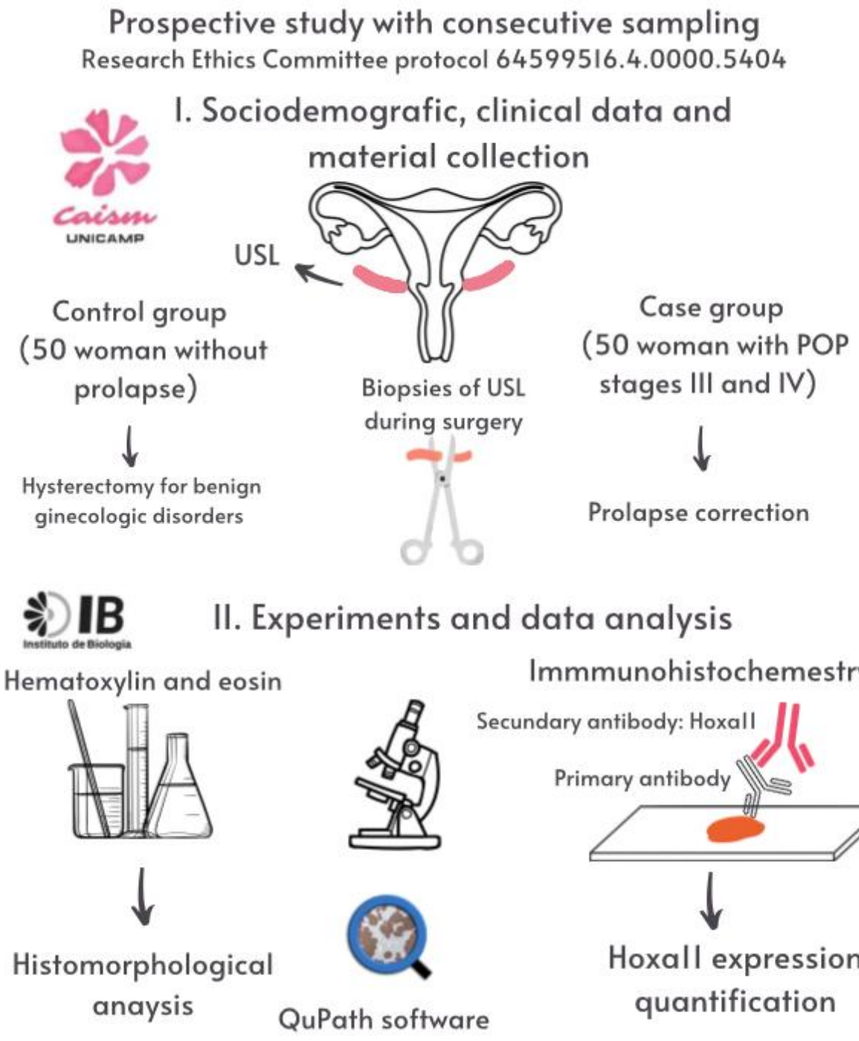
Hypothesis / aims of study

Pelvic organ prolapse (POP) results from the failure of the support mechanisms of the pelvic viscera, resulting in vaginal protrusion and the fall of the pelvic organs through the vaginal canal. The pathophysiology of genital prolapse is multifactorial, in which environmental factors such as lifestyle, parity, and childbirth interact with molecular, endocrine, and genetic factors. The uterosacral ligaments (USL) are composed of collagen, smooth muscle, elastin and nerve bundles. On POP there are histomorphological changes characterized by decreased smooth muscle content, decreased cellularity, alterations in the extracellular matrix (ECM), increased apoptosis, increased inflammation and increased adipocytes. The ECM dysfunction is characterized by alterations in metabolism and distribution of the main proteins, like changes in the proportions of collagen subtypes.

Hoxa11 is responsible for development of the female reproductive system and formation of uterosacral ligaments, lower uterine segment, and cervix, and also acts in collagen type III synthesis and matrix metalloproteinase 2 (MMP2) synthesis. HOXA11 regulates morphology and integrity of USLs by promoting cell proliferation and attenuating apoptosis and also regulating extracellular matrix homeostasis. Studies have shown reduced expression of HOXA11 associated with reduced expression of collagens, ECM disfunction and low cellularity in women with POP.

It is our hypothesis that due to the low expression of HOXA11 in women with prolapse, there are changes in the uterosacral ligament, as well as the organization of the connective tissue, proportion of smooth muscle and cellularity. Therefore, the objective of this study is to analyze the expression of the HOXA11 gene and its association with the histomorphological alterations in the uterosacral ligament in women with pelvic organs.

Study design, materials and methods



Results and interpretation

Variables	Control (n=50)	Case (n=50)	p-value
Age (mean ± standard error)	44.64 ± 6.45	65.94 ± 10.41	<0.001
BMI (mean ± standard error)	29.53 ± 4.78	28.02 ± 4.21	0.09
Pregnancies (mean ± standard error)	2.16 ± 1.27	4.68 ± 2.45	<0.001
Deliveries (mean ± standard error)	1.94 ± 1.11	4.1 ± 2.29	<0.001
Vaginal deliveries (mean ± standard error)	0.88 ± 0.35	3.6 ± 0.73	<0.001
Previous gynecological surgeries	7	22	0.001
Arterial Hypertension	14	30	0.001
Diabetes Mellitus	6	14	0.046
Other comorbidities	17	28	0.027
Medication use	32	44	0.005
POP Family history	6	14	0.046

Table 1. Clinical, sociodemographic, and patient history characteristics.

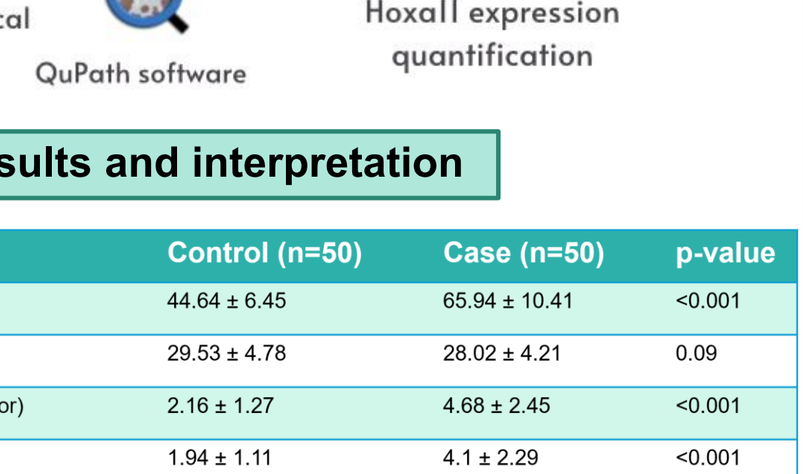


Figure 2. Representative photomicrographs of the uterosacral ligament from patients from the control group (**A** and **B**) and case group (**C** and **D**). Note the prevalence of dense connective tissue on control images and the prevalence of dense connective tissue on case images. Hematoxylin and eosin staining.

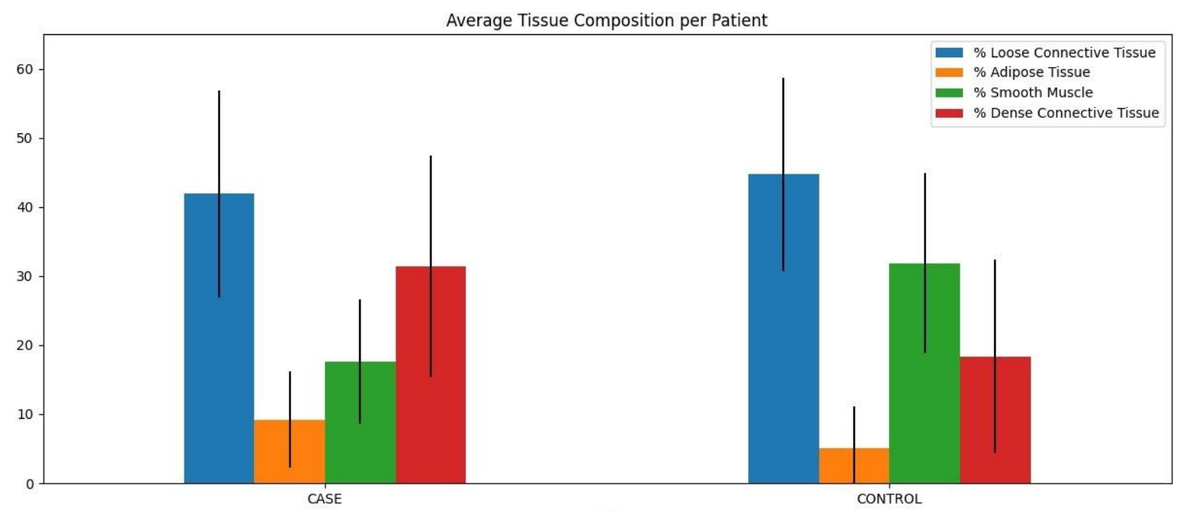


Figure 3. Analysis of USL tissue composition between CASE (n=11) and CONTROL (n=12). The CASE group presented significantly decreased Loose Connective Tissue (p=0.02), decreased Smooth Muscle (p=0.03) and increased Dense Connective Tissue (p=0.02). The CASE group revealed not significantly more Adipose Tissue (p=0.06).

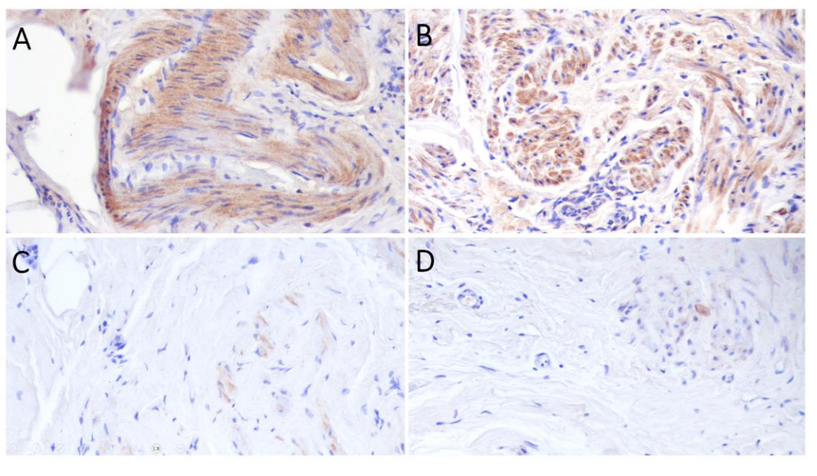
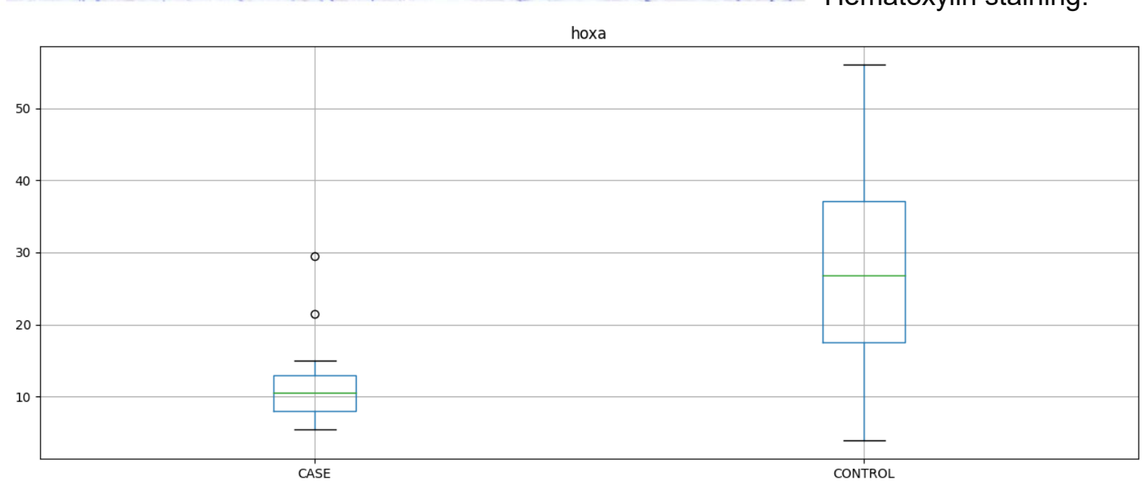


Figure 1. Representative photomicrographs of the control group (A and B) and case group (C and D) for immunostaining of HOXA-11. Note the strongly marked protein expression of HOXA11 in the control group and significantly reduced in the prolapse group. Immunohistochemistry with DAB revelation and Harry's Hematoxylin staining.



Graph 1. Representation of the quantitative analysis of Hoxa11 expression by immunohistochemistry. The CONTROL group (n=12) presented a 27.4% mean of Hoxa11 immunostained area, while the CASE group (n=11) presented a mean of 10.20% immunostained area (p=0.01).

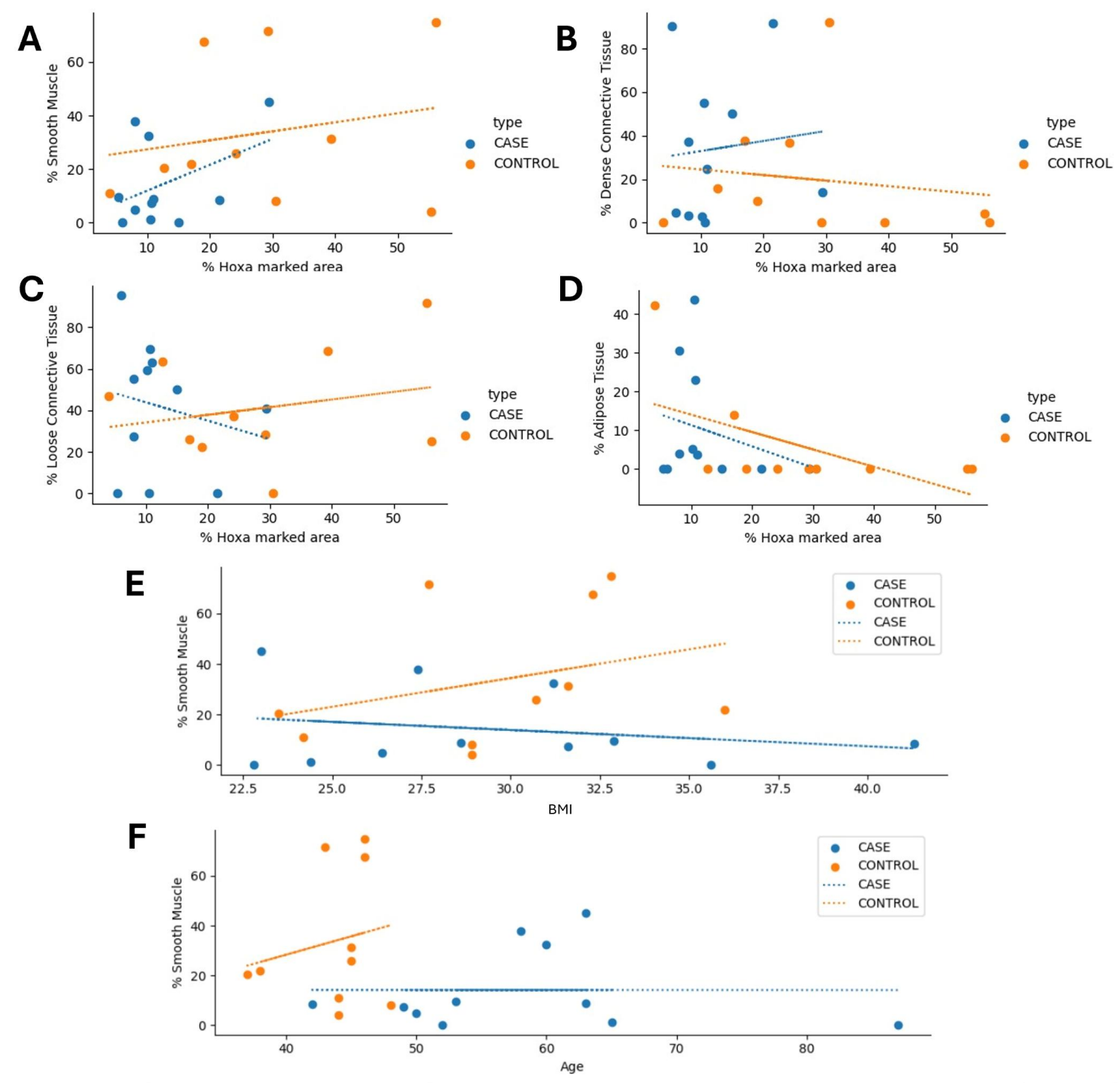


Figure 4. Correlation between USL tissue composition and Hoxa11 expression (**A, B, C, D**) and correlation between USL tissue composition and risk factors (**E, F**). Positive relationship was found between Smooth Muscle content (**A**) and Hoxa11 expression in both groups. The CASE group showed a negative correlation between Loose Connective Tissue (**B**) and Hoxa expression while the CONTROL group revealed positive correlation. Adipose Tissue content (**D**) revealed a negative relationship to Hoxa11 expression in both groups. Increase in BMI and ageing does not reflect on tissue disfunction with loss of Smooth Muscle on the CONTROL group, but affects CASE group (**E, F**).

Conclusions

Women with genital prolapse have a more frequent family history of prolapse, older age, higher parity, more previous surgeries, more comorbidities. HOXA11 gene expression is decreased in women with POP by IHC. Decreased HOXA11 expression is associated with USL tissue alterations. USLs in POP have extracellular matrix disorder with prevalence of loose connective tissue and reduction of dense connective tissue and reduction of smooth muscle.