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Expression and importance of relaxin in vaginal wall tissues from women with pelvic organ prolapse and with/without stress urinary incontinence

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Abstract

Background and aim: Thousands of women worldwide suffer from pelvic organ prolapse and stress urinary incontinence. Low quality of pelvic connective tissue contributes to the development of the above conditions. Previous studies suggest that relaxin reduces the tensile strength of pelvic connective tissue. The aim of this study was to assess the presence of relaxin in uterine supporting tissues from patients with pelvic organ prolapse and stress urinary continence.

Methods: We recruited 90 women: 30 patients with pelvic organ prolapse and stress urinary incontinence, 30 continent women with pelvic organ prolapse and 30 controls. Tissue samples from the participants were obtained from the insertion sites of the uterosacral and cardinal ligaments at the time of hysterectomy. Immunostaining defined relaxin expression intensity.

Results: There are statistically significant differences regarding the intensity of relaxin expression between women with pelvic organ prolapse plus stress urinary incontinence and continent women with or without pelvic organ prolapse, as well as between women with prolapse plus incontinence and controls ($P<0.05$). Continent women with prolapse and controls were more likely to have negative relaxin expression than incontinent women with prolapse. Not any statistically significant differences were found between women with both prolapse and incontinence and women with prolapse only, as well as between continent women with prolapse and controls. Incontinent patients with prolapse had a higher probability of having positive relaxin expression than controls. No women with prolapse and only one control had strongly positive relaxin expression.

Conclusions: Positive relaxin expression in pelvic connective tissue could be a predisposing factor for pelvic organ prolapse and/or stress urinary incontinence. Possible future interventions based on relaxin expression may aid in the non-surgical management of pelvic organ prolapse and stress urinary incontinence.

Introduction

Pelvic organ prolapse is a stressful situation for women, which increases with age and depends on many factors, especially pregnancy and childbirth trauma. This condition becomes unbearable when combined with stress urinary incontinence, making women functionally disabled and downgrading their quality of life. These situations coexist at a rate of more than 50% of cases [1]. Two thirds of the prevalence of stress urinary incontinence is borne by women and it is inclined that this happens mainly due to the female pelvic anatomy, pregnancy and the neuromuscular damage that arises from vaginal childbirth, without overlooking the genetic predisposition of women [2]. Pelvic support is based on functionality of extracellular matrix (ECM) in the connective tissue and is a mixture of long chain proteins, including collagen, elastin, and proteoglycans.

The ECM produced in most connective tissue cells is composed of two major materials: glycozaminoglycanes (GAG) and fibrillar proteins. In addition, there are small amounts of structural glycoproteins present in the extracellular matrix with important roles in cell adhesion, such as relaxin, tenascin and fibronectin [3].

The ECM is maintained by fibroblasts through the secretion of protein and growth factors, which form the composition and decomposition of the building blocks which support the pelvic floor.

The purpose of this study was to demonstrate the intense presence of relaxin in the supporting tissues of the uterus from postmenopausal patients with pelvic organ prolapse and stress urinary continence or with pelvic organ prolapse without stress urinary incontinence, suggesting

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a possible role of the relaxin system in the pathophysiology of pelvic organ prolapse and its relationship to stress urinary incontinence, and indicating the above system as a potential therapeutic target for pharmacological treatment of pelvic floor relaxation.

Materials and methods

The study was performed at the Second Department of Obstetrics and Gynaecology of National and Kapodistrian University of Athens Medical School in "Aretaieio" University Hospital. Approval for this study was obtained from the Institutional Ethics Committee for Research involving human subjects (reference number B-177/31-05-2016). The study has followed all standards set by the 1964 Declaration of Helsinki of the World Medical Association General Assembly.

We included 90 white adult Greek postmenopausal women recruited between 18th October 2016 and 31st October 2019. The participants were categorised into three groups: two study groups and one control group (n=30 each). All 90 women gave written informed consent. The patients were referred from the Outpatient Urogynaecology Unit of our Department and were asked to take part in the present study following admission to our hospital. The first study group (Group A) comprised 30 women with both stress urinary incontinence (SUI) and symptomatic pelvic organ prolapse (POP) [Stages I, II, III or IV; POP quantification (POP-Q) system]. The second study group (Group B) included 30 women with symptomatic POP (Stages I-IV; POP-Q system), but without SUI. The control group (Group C) consisted of 30 women without SUI and without POP (Stage 0; POP-Q system. Stage 0 is the gold standard for normal pelvic organ support). The 60 women of Groups A and B underwent total vaginal hysterectomy either as a sole procedure or as part of a more extensive pelvic reconstructive operation, for example total vaginal hysterectomy combined with anterior colporrhaphy and posterior colpoperineorrhaphy. The 30 women of Group C underwent total abdominal hysterectomy for a variety of benign conditions other than POP. Women with a personal medical history of urogenital malignancy, endometriosis, systemic autoimmune disease(s), chronic obstructive pulmonary disease, previous pelvic surgery, and previous usage of oestrogen or progesterone supplementation were excluded from the study.

The initial gynaecological examination and clinical evaluation was performed by the Directing Professor of the Urogynaecological Unit of our Department. All participants were examined in a supine position with a full urinary bladder and were asked to perform a Valsalva manoeuvre. The furthest descent of the vaginal walls was measured at maximum strain effort using the standardised "IUGAstix for POP-Q" by the International Urogynaecological Association. Total vaginal length was measured both in rest and in maximum stress using single-use plastic Sims bivalve speculum. Each patient was also examined immediately afterwards in standing position, to fully determine the exact degree of prolapse. Furthermore, each participant was asked about her age, her Body Mass Index (BMI) and her smoking habits.

Tissue samples from the participants were obtained from pubocervical fascia and from the insertion sites of the uterosacral and cardinal ligaments of the uterus right after hysterectomy, using Metzenbaum surgical scissors. The samples were afterwards immediately transported to the Pathology Laboratory of our hospital for immunostaining for relaxin. These were then fixed in neutral-buffered 5% formalin solution and subsequently soaked in paraffin blocks. Following routine processing, 0.4 mm paraffin slices were stained with haematoxylin-eosin stain and studied under a classic Zeiss™ classical photomicroscope. Additional slides were studied with immunohistochemistry after processing in a Ventana BenchMark ULTRA IHC System™ with monoclonal antibodies special for relaxin (Relaxin R1/ILGR7, monoNBP2/23674). Evaluation of immunostaining was carried out by a semi-quantitative method as follows:

- Negative (-), when less than 10% of stromal cells showed positive immunostaining.
- Weakly positive (+), when 10 to 20% of stromal cells showed positive immunostaining.
- Strongly positive (++), when more than 30% of stromal cells showed positive immunostaining.

The statistical analysis of the sample's quantitative and qualitative parameters was carried out by a specialised experienced biostatistician with SPSS™ statistical software, version 25 (IBM Corporation, Armonk, New York, United States of America). Regarding descriptive statistics, absolute frequencies and relative frequencies were used to analyse demographic and experimental data and were summarized as numbers with percentage. The comparison of the various relaxin intensity of expression frequencies between patients and control subjects was performed using the Chi-square test for independence in a 3x3 contingency table. The quantitative variables of the present study, i.e. age and BMI, did not follow the normal distribution, because all three groups failed to pass the Kolmogorov-Smirnov normality test. Therefore, the non-parametric Mann-Whitney U test was used for the differences of means and medians. The possible statistical significance between the remaining (categorical) variable, i.e. smoking, was tested using the Chi-square test. A p-value of less than 0.05 was considered significant.

Results

There were no statistically significant differences between the groups of patients (Groups A and B) and the group of controls (Group C) regarding age, Body Mass Index (BMI) and smoking ($P>0.05$), as shown in Table 1.

Moreover, Table 2 shows that there are statistically significant differences regarding the intensity of relaxin expression between women with POP and SUI and continent women either with POP or without POP, as well as between women with both POP and SUI and healthy controls ($P<0.05$). Continent women with POP and controls were more likely to have negative relaxin expression than incontinent women with POP.

However, not any statistically significant differences were found between women with both POP and stress urinary incontinence and women with POP only, but without SUI, as well as between continent women with POP and controls. Group A patients had a higher probability of having positive relaxin expression than Group C women. No women with POP and only one control had strongly positive relaxin expression.

Discussion

Relaxin is a heterodimer protein hormone of two peptide chains, which appears related to insulin and structurally similar to both Nerve Growth Factor (NGF) and Insulin-like Growth Factor (IGF) [4,5]. In the human female, relaxin is mainly produced in the corpus luteum of the ovary, in the connective tissue of the breast and in the placenta, chorion and decidua. This hormone seems to have several roles and

Table 1. Demographic parameters of patients and controls

		Group			p-value					
		A	B	C	Total	All groups	A-(B+C)	A-C	A-B	B-C
Parameter										
	41-60	12 (40%)	13 (43.3%)	13 (42.2%)	38 (42.2%)					
Age	61-70	10 (33.3%)	10 (33.3%)	9 (32.2%)	29 (32.2%)	1	1	1	1	1
	>71	8 (26.7%)	7 (23.3%)	8 (25.6%)	23 (25.6%)					
	Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)					
	18-25	21 (70%)	22 (73.3%)	21 (70%)	64 (71.1%)					
BMI	25-29	8 (26.7%)	7 (23.3%)	8 (26.7%)	23 (25.6%)	1	0.911	1	1	1
	>29	1 (3.3%)	1 (3.3%)	1 (3.3%)	3 (3.3%)					
	Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)					
	Yes	12 (40%)	14 (46.7%)	13 (43.3%)	39 (43.3%)					
Smoking	No	18 (60%)	16 (53.3%)	17 (56.7%)	51 (56.7%)	0.963	0.822	1	0.795	1
	Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)					

Table 2. Intensity of relaxin expression in patients and controls

		Group			p-value					
		A	B	C	Total	All groups	A-(B+C)	A-C	A-B	B-C
Relaxin										
	-	7 (23.3%)	14 (46.7%)	17 (56.7%)	38 (42.2%)					
Expression Intensity	+	23 (76.7%)	16 (53.3%)	12 (40%)	51 (56.7%)	**0.021**	**0.015**	**0.008**	0.103	0.438
	++	0	0	1 (6.7%)	1 (1.1%)					
	Total	30 (33.3%)	30 (33.3%)	30 (33.3%)	90 (100%)					

target a variety of tissues, such as the endometrium, the myometrium, the cervix uteri, the breast, as well as skin connective tissue, pubic symphysis, and pelvic ligaments [6]. Furthermore, relaxin receptors have been identified in the heart and the nervous system [7]. Relaxin induces matrix metalloproteinase (MMP) transcription in the cardiovascular system [8]. Therefore, relaxin plays a significant role in regulating extracellular matrix turnover and mediates its action through the G-protein receptor relaxin family peptide receptor 1 (RXFP1) [9]. Relaxin receptor LGR7/RXFP1 mRNA is also detected in human kidney, adrenal gland, heart and brain, which implies further roles for relaxin in the renal-cardiovascular, central nervous and autonomous nervous system [7,10]. Relaxin isoforms 1 and 2 reconcile the haemodynamic physiologic alterations which establish during pregnancy, such as cardiac output, renal blood flow and arterial compliance [11]. Consequently, relaxin has been suggested as potential drug for the treatment of hypertension, for the prevention of preeclampsia and as a novel treatment for congestive heart failure [12].

Relaxin in mammals displays several roles, such as facilitation of follicle rupture at ovulation, facilitation of blastocyte implantation, foetal growth, uterine growth and accommodation, uterine stromal remodelling during pregnancy, inhibition of spontaneous myometrial activity during pregnancy, facilitation of foetal membrane rupture, cervical ripening and facilitation of parturition, and mammary growth during pregnancy [5]. Moreover, relaxin relaxes pelvic ligaments and is believed to soften the pubic symphysis, especially during pregnancy [13]. Relaxin is important for the modification of the pelvic connective tissue to facilitate foetal delivery. Therefore, it is supposed to have a connective tissue remodelling effect [14].

Pelvic organ prolapse prevails in women with increasing age and it is estimated that affects almost every second elderly woman [15]. Neuromuscular damage from the mechanical stretch of vaginal delivery and the bad quality of pelvic connective tissue support contribute to the aetiology of stress urinary incontinence and pelvic organ prolapse [2]. Hormonal factors have also been suggested to contribute to the development of the above diseases [15]. Changes of the pelvic connective tissue might promote the progression of pelvic organ prolapse, since rectus fascia from pregnant women was found to have less tensile strength in comparison to fascia from non-pregnant women [14]. Relaxin probably acts as a paracrine signalling molecule in women and thus peripheral serum levels may not always affect its activity. Although little increased joint mobility is necessary for labour and delivery, some women experience pelvic girdle relaxation to a much greater extent. This is more common in women of Scandinavian origin, which implies a possible role for genetics. High relaxin levels or greater susceptibility to relaxin may contribute to this problem [6]. In a cohort study of Kristiansson *et al.* [14] which included 200 women in early pregnancy, high serum relaxin levels were associated with joint laxity and pelvic pain in pregnancy. Those subjects with the highest relaxin levels had the greatest recovery time after pregnancy.

Despite the above, the relation between relaxin and pelvic organ prolapse has not been clearly described [16]. A functional extracellular matrix is prerequisite for pelvic support [2]. Relaxin can interfere with connective tissue metabolism at many levels, including the inhibition of profibrotic factors (like Transforming Growth Factor-beta, TGF-β), the activation of MMP-mediated extracellular matrix degradation and the inhibition of fibroblast proliferation and differentiation [12]. Relaxin seems to activate the system of collagenolysis, control new collagen formation and changes the ground substance by lowering the viscosity and raising the water content. This multiple action decreases the intrinsic strength of the connective tissue, enabling it to expand and loosen [6]. In addition, uterosacral ligaments (USL) are regarded as possible relaxin targets. These ligaments mainly consist of collagenous connective tissue, smooth muscle in the ground reticulum and blood vessels, thus they have both dynamic (smooth muscle) and

static (collagen fibres) properties which are necessary for pelvic floor suspension. The active tone of the smooth muscle part is regulated by autonomic nerves and circulating stimulants, such as hormones. Collagenous connective tissue is responsible for the passive tone of the USL. Alterations in collagen and smooth muscle contents have been reported in the USL of women with POP. Relaxin binds with high affinity to and activates the LGR7 receptor as signal transducer not only in USL, but also in smooth muscle (SMC) and stromal cells, leading to loss of ligament strength via supposed stromal remodelling effects and low contractility of SMC. The upregulated activity of MMPs in USL of patients with POP can also be activated by incubation with relaxin in human fibroblasts of the lower part of the uterus [12,15]. Therefore, the relaxin system could be a potential target for a non-surgical treatment of POP [1].

There have been reported conflicting results on the association between serum relaxin and female urinary incontinence (UI) [16]. Relaxin presumably facilitates UI by degrading the collagen of the pelvic floor's connective tissue. However, it has been shown that women who had been continent throughout pregnancy had statistically significant higher serum relaxin levels than incontinent women. Relaxin might favour continence by either stimulating tissue growth in the lower urinary tract and, thus, leading to elevated urethral pressure, or by its suggested vasodilatory effect on the microcirculation, which could also increase urethral pressure. Relaxin could also have a more mechanical effect by remodelling the connective tissue of the bladder neck region. As a result, this region becomes less rigid and micro-ruptures are prevented as the pregnant uterus expands [14].

The results from our study come in agreement with previous studies and confirm that the associations between relaxin expression, pelvic organ prolapse, and stress urinary incontinence are not clearly understood and require further investigations in various populations. Indeed, in our study's sample, women with POP and SUI were more likely to have a weak relaxin expression than healthy controls, who were more likely to have negative relaxin expression. Moreover, the group of women with POP and SUI was more likely to have a weak relaxin expression rather than negative relaxin expression. Relaxin did not seem to greatly contribute to the development of SUI, as for women with (both weak and strong) positive relaxin expression, incontinent were significantly less than continent ones, regardless of their prolapse status. It is also interesting that only one woman had strong relaxin expression and this woman belonged to the healthy controls.

The present study is limited by the fact that relaxin measurements were semi-quantitative. However, relaxin was measured directly from tissues and not from peripheral blood samples. Moreover, the sample size does not warrant the validity of conclusions in comparison to a bigger sample or a different population and our results were not compared with results of peripheral whole venous blood samples from the same subjects.

Conclusions

Relaxin is a hormone with a vast spectrum of actions and a wide variety of confirmed and potential targets. These characteristics, in combination with its availability for clinical trials, make the study of relaxin an exciting field for future research. Despite the conflicting clinical data on the role of relaxin in the development of stress urinary incontinence and pelvic organ prolapse, possible therapeutic interventions related to relaxin may prove to be revolutionary, such as in the non-surgical treatment of pelvic organ prolapse.

Conflicts of interest

None

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