

Comparison of IGF-1 Protein Expression as a Marker for Type I Collagen Production in the Uterosacral Ligament of Patients with Uterine Prolapse and without Uterine Prolapse to Prevent Uterine Prolapse

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Abstract

Objective: This study aims to investigate the risk factors associated with the lower IGF-1 protein expression in the uterosacral ligament in relation to the incidence of uterine prolapse. By identifying the relationship between IGF-1 protein expression in the uterosacral ligament and the incidence of uterine prolapse, it is anticipated that this study will provide a basis for the early identification of uterine prolapse.

Method: This study utilized a cross-sectional immunohistochemistry method for IGF-1 protein on 34 pelvic organ prolapse (POP) subjects and 34 controls at RSUP Dr. Hasan Sadikin, Bandung, in 2024. A sample is taken from the distal portion of the uterosacral ligament, prepared into paraffin blocks and underwent immunochemistry staining. It was then reviewed and IGF-1 protein expression graded by a consultant pathologist.

Results: IGF-1 protein expression in the uterosacral ligament was found to be lower in uterine prolapse patients (168.2 ± 3.1) compared to those without uterine prolapse (181.2 ± 13.3) ($p=0.002$).

Conclusion: IGF-1 levels in uterine prolapse are lower than those without uterine prolapse.

Keywords: IGF-1, collagen type I, uterosacral ligament, uterine prolapse

Perbandingan Ekspresi Protein IGF-1 sebagai Penanda Produksi Kolagen Tipe I pada Ligamentum Sakrouterina Penderita Prolaps Uterus dan Tanpa Prolaps Uterus untuk Pencegahan Prolaps Uterus

Abstrak

Tujuan: Penelitian ini bertujuan untuk meneliti faktor risiko rendahnya ekspresi protein IGF-1 pada ligamentum sakrouterina terhadap kejadian prolaps uterus. Dengan diketahui adanya hubungan antara ekspresi protein IGF-1 pada ligamentum sakrouterina dengan terjadinya prolaps uterus, diharapkan penelitian ini dapat berguna sebagai acuan untuk identifikasi prolaps uterus lebih dini.

Metode: Penelitian ini menggunakan metode potong lintang imunohistokimia protein IGF-1 pada 34 subjek prolaps organ panggul (POP) dan 34 kontrol di RS Dr. Hasan Sadikin Bandung tahun 2024. Sampel diambil dari bagian distal ligamentum sakrouterina, dibuat blok paraffin, dan dilakukan pewarnaan histokimia. Ekspresi protein IGF-1 kemudian diperiksa dan dinilai oleh ahli patologi.

Hasil: Ekspresi protein IGF-1 di ligamentum sakrouterina pada penderita prolaps uterus lebih rendah ($168,2 \pm 3,1$) dibandingkan dengan tanpa prolaps uterus ($181,2 \pm 13,3$) ($p=0,002$).

Kesimpulan: Kandungan IGF-1 pada prolaps uterus lebih rendah dibandingkan dengan tanpa prolaps uterus.

Kata kunci: IGF-1, kolagen tipe I, ligamentum sakrouterina, prolaps uterus

Introduction

Uterine prolapse is a condition where the uterus descends into the vaginal cavity due to the weakening of the pelvic floor support structures. This condition can occur in all age groups of women and is generally not life-threatening, but it can cause significant morbidity affecting the psychosocial life, well-being, and sexual function of those affected.¹ Uterine prolapse is one of several conditions under the broader term of pelvic organ prolapse (POP).²

It is challenging to separate rates of uterine prolapse from POP as most studies group these two conditions together.² According to estimates by the World Health Organization, the global prevalence of uterine prolapse among women under the age of 45 ranges from 2% to 20%.³ In Indonesia, there is limited data regarding the incidence of uterine prolapse. According to the annual report of RSUP Dr. Hasan Sadikin Bandung in 2022, 2.25% of patient visits were related to uterine prolapse out of all gynecological cases. Meanwhile, at RSUP Sanglah Denpasar, uterine prolapse accounted for 11.38% of all gynecological cases in 2014–2015.^{4,5}

Those data showed the “iceberg phenomenon” of uterine prolapse—only a fraction of affected individuals seek medical attention, typically when symptoms become severe or disruptive. Many women do not seek medical help due to the absence of noticeable symptoms, social stigma, or a lack of awareness about POP and its consequences.⁶

Preventing uterine prolapse requires identifying risk factors and understanding its underlying causes. DeLancey classified the pelvic floor tissue into three structural levels: level I (endopelvic fascia), level II (pelvic diaphragm), and level III (urogenital diaphragm). Weakness at level II can increase the strain on the cardinal and uterosacral ligaments, which have a crucial role in

supporting pelvic organs. Additionally, weakness in the cardinal and uterosacral ligaments can contribute to uterine prolapse.⁷ Although the composition of these structures is pleomorphic, the distal portion of the uterosacral ligament is histologically rich in collagen and elastin but contains fewer muscle fibers. This makes the distal uterosacral ligament an interesting target for studying collagen composition changes in women with uterine prolapse.⁸

Among 28 known types of collagens, type I, III, and V are present in the vagina and the ligaments that support the pelvic organs. Type I collagen is the most abundant collagen in the body, with its flexibility and strength, and it is highly resistant to mechanical stress. Type III collagen is abundant in tissues requiring high flexibility and elasticity,⁹ while the function of Type V collagen in the vagina and supporting tissues remains unclear.¹⁰

Research suggests that reduced collagen levels in the pelvic floor, mainly type I collagen, is a key factor in weakening its supportive structures, increasing the risk of uterine prolapse. Ligament laxity caused by decreased collagen secretion can be prevented and treated by better understanding of the molecular mechanisms underlying uterine prolapse.¹¹

One of the molecular components involved in the progression of uterine prolapse is insulin-like growth factor (IGF-1), a polypeptide primarily synthesized in the liver and plays a vital role in extracellular matrix metabolism, cell proliferation, cell differentiation, and apoptosis.¹² A study has shown that IGF-1 levels in vaginal wall tissues are lower in patients with pelvic organ prolapse (POP) compared to those without POP.¹³ Additionally, declining IGF-1 protein expression in the vaginal walls of POP patients has been observed. IGF-1 helps maintain pelvic floor integrity by inhibiting autophagy and apoptosis, promoting expression of type I collagen, and regulating extracellular

matrix metabolism through activation of the Akt/mTOR/p70S6K pathway in vaginal fibroblasts.¹¹ Overall, IGF-1 stimulates fibroblast proliferation, enhances collagen synthesis, and helps prevent POP.

To date, no studies in Indonesia have explored the risk factors associated with low IGF-1 protein expression in the uterosacral ligament and its correlation with uterine prolapse. Understanding this correlation could serve as an essential reference for early identification of uterine prolapse, enabling preventive measures to be implemented more effectively.

Methods

This study design is a numerical analytic cross-sectional study. The sampling method was performed using a non-probability sampling technique, which consisted of consecutive sampling, meaning all eligible subjects who met the inclusion criteria were enrolled as samples until the required number of subjects was met. The inclusion criteria include patients who are willing to participate in the study, with uterine prolapse for the sample group, and patients without uterine prolapse who will undergo total abdominal hysterectomy for the control group. The exclusion criteria were patients with malignancies of the reproductive organs.

Once the required sample size was

determined, the uterosacral ligament tissue was taken according to the criteria. Sample labeling and sample registration were carried out using Microsoft Excel tools. The tissue is cut to the required size and inserted into a cassette that has been submerged in 10% formalin neutral buffer. The tissue is then processed to make paraffin blocks, and then immunohistochemical staining is continued. All research data is recorded on the form and processed statistically using the IBM SPSS Statistics program. Data distribution was assessed using the Shapiro-Wilk Test to ensure homogeneity for subsequent statistical analysis. To compare the mean of IGF-1 expression in the sacrouterine ligament between patients with uterine prolapse and without uterine prolapse, an analysis was performed with an unpaired t-test.

Results

This research was conducted at the Urogynecology & Reconstructive Outpatient Clinic, Department of Obstetrics and Gynecology, RSUP Dr. Hasan Sadikin, Bandung, from March 2024 to September 2024. During this time, all patients with and without uterine prolapse who underwent surgery and met the inclusion criteria were enrolled as research subjects. Thirty-four subjects were included in the sample group and 34 in the control group. Once data

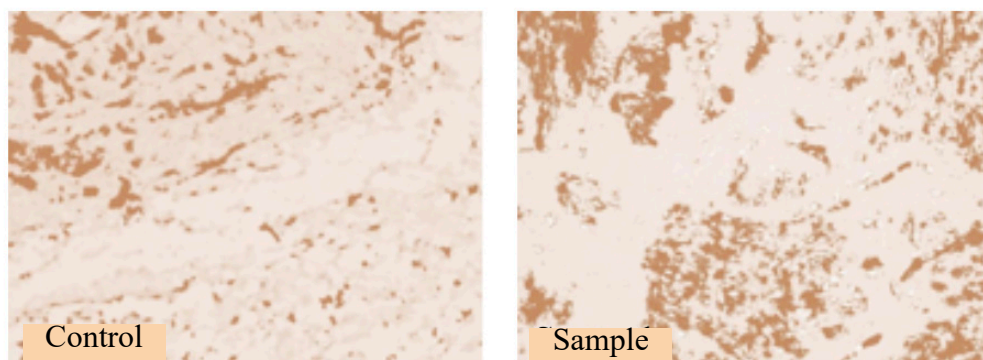


Figure 1 IGF-1 Expression in Uterosacral Ligament Tissue by Immunohistochemistry Staining

Table 1 Subject Characteristics

Characteristic	Uterine Prolapse (n=34)		Control (n=34)	
	Mean	SD	Mean	SD
Age (years)	57.21	5.30	58.03	4.64
Parity	3.26	1.54	2.59	0.89
BMI (kg/m ²)	23.96	2.02	23.92	2.04
Menopause Duration	14.15	7.09	4.12	4.78

Table 2. Comparison of IGF-1 Expression between Case and Control Groups

		Mean + SD		95% CI	p-value
		Case	Control		
IGF-1	Protein	168.2 + 3.1	181.2 + 13.3	(-20.6 - -5.37)	0.002
	Expression				

aGrouping variable: IGFCO

collection was completed, the data were evaluated and analyzed.

The intensity of immunohistochemistry (IHC) staining was measured across five fields of view and averaged for each sample. In the control group, staining intensity was lower, indicating higher IGF-1 protein expression, whereas in the research group, staining intensity was higher, suggesting lower IGF-1 protein expression.¹⁴

Table 1 shows the characteristics of the research subjects. A correlation test was carried out between all variables, and no statistically significant results were obtained ($p > 0.05$), which indicates that none of the variables statistically affect the expression of IGF-1 protein.

A comparison of IGF-1 protein expression in POP patients and the control group was done using the Mann-Whitney test. Statistically significant results were obtained ($p=0.002$), so it can be concluded that the expression of IGF-1 protein in the uterosacral ligament in patients with uterine prolapse is lower than without uterine prolapse.

Discussion

Risk factors of uterine prolapse were mainly divided into non-modifiable and modifiable

factors. Non-modifiable factors consist of age, race, genetic composition, and collagen depletion, while modifiable factors consist of occupation, obesity, smoking, and triggering risk factors such as childbirth that cause damage to the supporting tissue of the pelvic organs.¹⁵ Currently, among the all-recognized POP risk factors, the risk factors that play a significant role in POP are old age, multiparity, high BMI, and chronic straining.¹⁶

This study shows the mean age of women in the uterine prolapse case group was 57.21 ± 5.30 years, while in control groups, it was 58.03 ± 4.64 years. A study by Wu et al. stated that among symptomatic POP sufferers, 6% of them are 20-29 years old, 31% of them are 50-59 years old, and about 50% of POP sufferers are 80 years or older.¹⁷

The prevalence of POP in Indonesia is still uncertain. However, research in Surabaya found a significant difference between the degree of POP and age, with the majority of patients being older than > 50 years old.¹⁸ Another study showed that the prevalence of POP increases with age. The risk of POP increases by 10% for each decade of increasing patient age.¹⁹

The average parity of women in the case group of this study was 3.26 ± 1.54 , while in the

control group, it was 2.59 ± 0.89 . This result is in line with Bradley et al., who explained that as the number of parities increases, the pelvic organs will descend further into the vagina.²⁰ Another study mentioned that the risk of POP increased tenfold after vaginal delivery. During the labor process, neuromuscular damage not only causes direct injury to the levator but also damage to the nerve supply of those muscles, which can lead to the inability of the muscles to contract even though the muscles remain intact. When the pelvic floor muscles relax or are injured, the genital hiatus opens, and the suspensory ligament must support the pelvic organs. Although ligaments can withstand this load for a short time, the connective tissue will further stretch and eventually fail to support if the pelvic floor muscles do not close the pelvic floor in time.¹⁰

The average BMI in this study's case group was 23.96 ± 2.02 , while in the control group was 23.92 ± 2.04 . A study found that women who are overweight (odds ratio (OR) 2.51, 95% confidence interval 1.18-5.35) or obese (OR 2.56; 95% confidence interval 1.23-5.35) are at high risk of prolapse. Research conducted by Lee et al. concluded that increased BMI is an important lifestyle factor that affects pelvic prolapse. The most likely mechanism to cause the development of POP among obese women is increased intra-abdominal pressure, leading to weakness of the pelvic floor innervation, muscles, and fascia.¹⁷

Most subjects in the case group had entered menopause, with the average duration of menopause in the case group of 14.15 ± 7.09 , and in the control group was 4.12 ± 4.78 . The high incidence of postmenopausal POP suggests a strong correlation between declining estrogen levels and POP severity. During menopause, alteration in collagen concentration and quality, connective tissue morphology, and the role of estrogen in collagen metabolism are indicators of

estrogen's involvement in the occurrence of POP. The impact of estrogen on tissues depends not only on estrogen concentration but also on estrogen receptors. Estrogen receptors are found in various tissues, including in the nucleus of connective tissue cells, vaginal mucosa, levator ani muscle, and uterosacral ligament, which plays an important role in uterine support. Studies have shown that postmenopausal women with POP have significantly lower serum estrogen levels and reduced estrogen receptor expression in pelvic floor ligaments compared to those without POP.¹⁹

A statistical analysis was conducted in this study to compare IGF-1 protein expression between the two groups using the Mann-Whitney test. The results were statistically significant ($p=0.002$), indicating that IGF-1 protein expression in the uterosacral ligament was lower in patients with uterine prolapse than those without uterine prolapse. These findings align with a study by Yin et al. (2020), which demonstrated that compared to healthy subjects, IGF-1 protein expression was significantly reduced at both the mRNA and protein levels in vaginal wall tissue obtained from patients with POP. The study also revealed that decreased IGF-1 protein expression inhibited the viability of human vaginal fibroblast (HVF) cells, increased HVF cell apoptosis, and reduced the expression of Type I and III collagen. These effects were attributed to inhibiting IGF-1 receptor expression and suppressing the MAPK/NF- κ B signaling pathway.¹³ In addition, a study by Yin et al. (2021) demonstrated that the expression of type I collagen and IGF-1 was lower in the POP group than in the non-POP group. Additionally, type I collagen secretion was significantly reduced in the POP group. However, when IGF-1 was introduced to cells in the POP group, type I collagen secretion increased. The study also observed an upregulation of the LC3II/I autophagy marker, indicating an

enhancement of the autophagy process. Similarly, research by Yucen et al. reported a decrease in type I collagen expression in the uterosacral ligament of patients with POP.¹¹ Earlier findings by Chetty et al. highlighted IGF-1 role in collagen synthesis in fetal lung fibroblasts through the PI3K pathway.²²

Further studies have explored the role of IGF-1 in connective tissue integrity. Ozbek et al. found that IGF-1 protein expression was reduced in the serum of patients with urinary incontinence. Provenzano et al. reported that IGF-1 facilitates ligament tissue repair by increasing Type I collagen secretion. Additionally, Yan et al. demonstrated that periurethral IGF-1 injections promote the recovery of stress urinary incontinence (SUI) by stimulating myogenesis and revascularization.²³⁻²⁵

The collective findings from these studies suggest that reduced IGF-1 protein expression may impair ligament function, compromising pelvic floor support. Low IGF-1 protein expression in POP is closely linked to the aging process, childbirth, and menopausal status. As age increases, IGF-1 levels naturally decline, leading to weaker pelvic tissues and a reduced ability to maintain structural integrity, increasing the risk of prolapse. During childbirth, the pelvic floor undergoes significant stress, and repeated trauma can damage tissues, impair collagen synthesis, and reduce IGF-1 activity, making the pelvic organs more susceptible to prolapse. Additionally, menopause brings a decline in estrogen levels, which further reduces IGF-1 expression, compromising the strength and elasticity of pelvic tissues and contributing to the development of POP.

To date, no research in Indonesia has specifically examined the relationship between IGF-1 and POP. In this study, we observed decreased IGF-1 protein expression in the sacrouterine ligament of POP patients. However, a key limitation of this study is its cross-sectional design, which restricts the

ability to establish causality. Despite this, our findings suggest that IGF-1 plays a crucial role in the pathogenesis of pelvic floor dysfunction.

Conclusion

The results of this study showed that low IGF-1 protein expression in the uterosacral ligament is a risk factor for uterine prolapse.

Recommendation

Further research is needed to identify other factors contributing to the occurrence of uterine prolapse.

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