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Expression of insulin-like growth factor binding protein 5 in the vaginal wall tissues of older women with pelvic organ prolapse

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This study aimed to investigate the expression and significance of insulin-like growth factor binding protein 5 (IGFBP5) and extracellular matrix (ECM) related proteins in anterior vaginal wall tissues among aged pelvic organ prolapse (POP) patients. Tissues from the anterior vaginal wall were collected from 28 patients with POP and 20 patients without POP. The expression of protein and mRNA levels of IGFBP5 and ECM related proteins were evaluated in the vaginal wall tissues using immunohistochemistry, western blotting, and RT-qPCR techniques. The expression levels were then compared with clinical parameters. The expression levels of protein and mRNA of IGFBP5, collagen I, and collagen III were significantly lower in the POP group. Protein and mRNA expression levels of MMP2 were significantly higher in the POP group. IGFBP5 protein and mRNA expression levels were negatively correlated with age and significantly lower in older POP patients (≥ 65 years old) compared to younger POP patients (< 65 years old). IGFBP5 protein and mRNA expression levels were also significantly lower in POP-Q stage IV patients compared to POP-Q stage III patients. IGFBP5 expression level is negatively correlated with the age and severity of prolapse. The significant decrease in IGFBP5 expression may play a crucial part in the aging process and the occurrence of POP.

Keywords IGFBP5, ECM, Vaginal wall, Pelvic organ prolapse, Aging

Pelvic organ prolapse (POP) is a common and chronic disease characterized by the weakness of the supportive structure^{1–3}. The pelvic organs are maintained in proper anatomical position via a complex supporting structural system that is mainly composed of connective tissue. Connective tissue is composed of fibroblasts and their secreted extracellular matrix (ECM). Fibroblasts secreted and released collagen, contributing to the stability and adaptability of the pelvic floor. The degradation of collagen is carried out by a category of proteolytic enzymes known as matrix metalloproteinases (MMPs), while the function of MMPs is hindered by a category of proteins called tissue inhibitors of metalloproteinases (TIMPs). Recent researches suggest that changes in the connective tissue can cause damage to the pelvic floor support structures, thus contributing to the occurrence of POP^{4,5}. Already identified factors of POP include but are not limited to advanced age, menopause, obesity, pregnancy, vaginal delivery, estrogen deficiency, constipation, heavy lifting, chronic cough, and diabetes⁶. These factors may contribute to abnormal metabolism and remodeling of ECM in the pelvic floor, which subsequently affect their mechanical properties. Although there have been many researches on POP, little is known about its exact etiology and pathogenesis. Early prevention and treatment can be achieved through further research on the pathogenesis of pelvic organ prolapse.

Insulin-like growth factor binding proteins (IGFBPs) are secretory proteins that bind to IGFs or other ligands, and the IGFBPs exert both IGF-dependent and -independent effects⁷. IGFBP5 is the most conserved among the six IGFBPs and exhibits extensive biological activities including promoting cell survival, proliferation, senescence, differentiation, and apoptosis⁸. The localization of IGFBP5 within or outside of cells, specificity of the cell and tissue environment can influence the function of IGFBP5⁹. The levels of IGFBP5 expression were lower in aged adults than in young adults in the serum, bone, and skeletal muscle^{10,11}. Contrary to the downregulation

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of IGFBP5 due to aging, the upregulated expression of IGFBP5 is observed in both human and mouse aging thymus¹². A study has indicated that IGFBP5 can facilitate cell proliferation or apoptosis when cells undergo different contexts and conditions¹³. Nguyen's findings suggested that IGFBP5 is both an anti-angiogenic and pro-fibrotic factor⁷. IGFBP5 also plays a crucial role in regulating fibrosis and inflammation in various tissues¹⁴. IGFBP5 expression considerably increases and contribute to extracellular matrix deposition in fibrotic diseases, such as idiopathic pulmonary fibrosis and systemic sclerosis¹⁵. One study also confirmed that mice expressing human IGFBP-5 exhibited increased ECM gene expression to promote fibrosis¹⁶. Previous reports have examined the expression and biological activities of IGFBP5 in various tissues, however, there has been no research on exploring the relationships between IGFBP5 and POP.

IGFBP5 is associated with senescence¹⁷ and regulating fibrosis. In this study, we hypothesize that the decrease of IGFBP5 expression plays a crucial role in POP. The major objective of this research was to estimate the expression level of IGFBP5 in the vaginal wall among patients suffering from POP and explore whether IGFBP5 influences the occurrence and development of POP.

Materials and methods

Patient selection

Our investigation was performed following the Declaration of Helsinki. The protocol with code 2022PS154K was approved by the ethics committee of Shengjing Hospital of China Medical University. All the patients collected in a blinded, randomized fashion from February 2022 to August 2023. This study included 28 patients had advanced POP (stage III or IV in accordance with the POP-Q system) diagnosed by the same experienced expert and underwent pelvic floor reconstruction surgery, 20 patients without POP who had benign gynecological diseases and underwent total hysterectomy surgery. In order to rule out the impact of these diseases on the findings of this experimental, patients who had previous history of pelvic floor reconstruction surgery, connective tissue disorders, pelvic inflammatory conditions, hormone replacement therapy (HRT), gynecologic malignancies, endometriosis, and emphysema were excluded. 20 (aged 39–68 years) of the 28 (aged 39–82 years) POP patients were selected for the POP group and 20 (aged 48–73 years) patients without POP in the control group.

Tissue collection

During surgery, collected full-thickness vaginal wall tissue (approximately 0.5–1 cm²) from the obviously prolapsed region of the anterior vaginal wall near the vaginal apex of the POP patients and anterior vaginal wall near the vaginal apex of the control patients. Each specimen was divided into pieces. One piece was treated with formalin and embedded in 10% neutral-buffered paraffin for Masson's trichrome, HE, and immunohistochemical analyses. While the other piece was stored at –80 °C for RNA and protein analyses. Sometimes tissue was used only for one purpose due to some variability in the size of the sample.

Hematoxylin and eosin (HE) and Masson's trichrome staining

The vaginal specimens from both POP and control groups were sectioned longitudinally and put in 10% neutral buffered formalin for 2 days. They were cut into slides that were 4 µm thick, and placed onto coated slides after embedded in paraffin. The slides were subjected to the temperature at 60 °C for 120 min, deparaffinized, and were gradually rehydrated utilizing different concentrations of alcohol. They were then stained with HE and Masson's trichrome kit (Coolaber, SL7230, Beijing, China).

Immunohistochemistry

The UltraSensitive SP IHC kit (Maixin Biotech, Kit-9710, Fuzhou, China), was employed to conduct immunohistochemistry. After heating for 120 min at a temperature of 60 °C, deparaffinized, and rehydrated in gradient concentration alcohol. The slides were boiled in EDTA buffer for antigen retrieval in accordance with manufacturer. At room temperature, the sections were incubated for 10 min with 3% H₂O₂, blocked for 10 min with 5% goat serum. Subsequently, incubated with primary antibodies overnight at 4 °C. Next, sections were incubated for 10 min with biotinylated secondary antibodies at room temperature, and incubated for 10 min with streptavidin peroxidase at room temperature. After each step, specimens underwent PBS washing. Immune reaction was visualized using the DAB-ZLI-9017 kit (ZSGB-Biotech, Beijing, China). Finally, the slides underwent water washing prior to applying hematoxylin as a counterstain. Negative controls were treated with PBS instead of the primary antibody. The antibodies used are shown in Supplementary Table 1. Images were obtained utilizing a Nikon microscope (Nikon E100 Optical, Tokyo, Japan). The slides were examined using light microscopy at a magnification of 400×, maintaining the same camera settings. Selected five regions for every slide randomly. The positive area percentage and staining intensity were analyzed in all regions of the tissue (mean density = IOD sum/area sum) using ImageJ software (NIH, Bethesda, MD, USA). The average value of the selected five regions was utilized to quantify the level of target protein on each slide. Regions containing vessels were excluded from the analysis.

Western blotting

Cutting off the mucosal and muscular layers of anterior vaginal wall, total proteins were extracted by lysing the lamina propria tissue of vaginal samples with RIPA lysis buffer (Seven Biotech, SW104, Beijing, China) as instructed by the manufacturer. Measured the proteins utilizing the Omni-Easy™ Instant BCA Protein Assay Kit (Epizyme Biomedical Technology, ZJ102, Shanghai, China). Then, same amounts of protein from each sample were separated after subjected to SDS-PAGE. The separated proteins were transferred onto PVDF membranes using a current of 200 mA at 4 °C for 1–3 h. The membranes were blocked with a solution containing 5% non-fat milk at room temperature for 2 h. Subsequently, the membranes were incubated with primary antibodies at

4 °C overnight. The primary antibodies used are shown in Supplementary Table 2. Afterwards, the membranes were incubated with goat anti-rabbit secondary antibodies (1:3000, SA00001-15, Proteintech, USA) at room temperature for 1 h. After each antibody incubation step, unbound antibodies underwent TBST washing five times. The enhanced chemiluminescence (ECL) (MCE, Shanghai, China) detection method was used to visualize target bands. Beta actin served as an internal control. ImageJ software was employed to determine the density of all bands. A proportion of the target protein to the internal reference protein was used to express values of target protein.

Reverse transcription and quantitative real-time PCR

Total tissue RNA was obtained from the lamina propria of the anterior vaginal wall using TRIzol reagent (Seven Biotech, SM139-02, Beijing, China) as instructed by the manufacturer. The quality and quantity of total RNA were determined making use of the Nano-300 (All SHENG, Hangzhou, China) and cDNA synthesis was carried out using a reverse transcription kit (TransGen Biotech, AU341, Beijing, China) (1 µg of total RNA and in a reaction volume of 20 µl): 55 °C for 5 min, 85 °C for 5 s, then remained at 4 °C. RT-qPCR assays were performed on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) making use of the PerfectStart[®] Green qPCR SuperMix kit (TransGen Biotech, AQ601, Beijing, China). Sangon Biotech (Shanghai, China) synthesized the primers. Primer sequences are provided in Table 1. The reactions were conducted in triplicate. The reactions followed a protocol of 30 s at 94 °C, followed by 40 cycles of 5 s at 94 °C and 34 s at 60 °C, with a melting curve in the end. Data was analyzed using the relative quantitative method and GAPDH was used as the internal control. Three replicates being performed for each sample. The relative expression of each target gene was determined using 2^{-ΔΔCt} method.

Statistical analysis

The statistical software SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) was utilized for conducting the statistical analysis. Data were analyzed with independent sample t-test for continuous data with normal distributions, Mann–Whitney test for data with non-normal distributions, and chi-squared test for categorical data. Normal continuous variables were expressed as mean ± standard deviation (SD), while non-normal continuous variables were reported as median (interquartile range). Categorical variables were presented as numbers and proportions. GraphPad Prism 9.3.0 (GraphPad Software, Inc., La Jolla, CA, USA) was utilized for graphical plotting. Variations were regarded as statistically significant when $P < 0.05$.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Shengjing Hospital of China Medical University (No. 2022PS154K).

Consent to participate

Informed consent is not necessary to obtain in this study because the tissues are abandoned in surgery the informed consent is exempted by the ethics committee of Shengjing Hospital of China Medical University.

Results

Baseline characteristics of patients

In Table 2, the baseline characteristics of the patients in control group and POP group are showed. No noteworthy disparities were noted between the two groups in the way of age, body mass index (BMI), menopausal status, cesarean rate, parity, or gravidity ($P > 0.05$); the two groups were comparable. Menopause is medically characterized by the absence of menstruation for a minimum duration of 1 year.

Histologic and immunocytochemical staining

Hematoxylin–eosin (HE) staining demonstrated the features of histology of the intact vagina, including four anatomical layers: stratified squamous epithelial, lamina propria, muscularis, and adventitia (as shown in Supplementary Fig. 1). Figure 1 displays representative images for each stain. The localization of the majority of

Gene	Primer sequences
IGFBP5	Forward ACCTGAGATGAGACAGGAGTC Reverse GTAGAATCCTTTGCGGTCACAA
Collagen I	Forward AGGGCCAAGACGAAGACATC Reverse AGATCACGTCATCGCACACA
Collagen III	Forward TGGCTACTTCTCGCTCTGCTT Reverse CGGATCCTGAGTCACAGACACA
MMP2	Forward AGTTTCCATTCCGCTTCCAG Reverse CGGTCGTAGTCCTCAGTGGT
TIMP1	Forward CATCACTACCTGCAGTTTTGTG Reverse TGGATAAACAGGAAACACTGT
GAPDH	Forward CAGGAGGCATTGCTGATGAT Reverse GAAGGCTGGGCTCATT

Table 1. Primer sequences of each gene.

Baseline characteristics	Control group (n = 20)	POP group (n = 20)	P
Age, years	57.00 ± 7.108	61.05 ± 6.878	0.075
BMI, kg/m ²	23.26 ± 3.029	24.61 ± 3.396	0.192
Gravity, per child	3 (2–3.75)	2 (1–3)	0.066
Parity, per child	1.5 (1–2)	1 (1–1.75)	0.130
Menopause rate, %	70 (14)	90 (18)	0.107
Cesarean rate, %	20 (4)	5 (1)	0.139
Smoking habit, %	0 (0)	0 (0)	–

Table 2. Patient baseline characteristics. Data are presented as mean ± standard deviation (SD), median (interquartile range), or percentage (n) according to the situation; $P < 0.05$ was considered statistically significant. BMI, body mass index.

collagen within the lamina propria was showed through HE, Masson's trichrome, and immunocytochemical staining. Staining revealed presence of discoloration between the muscle bundles in the layer of muscularis, while no collagen immunostaining was identified in the stratified squamous epithelial layer. In relation to the control group, the appearance of collagen fibers in the lamina propria layer were much more discontinuous, loose, and disordered in the POP group, as depicted in Fig. 1.

Immunohistochemistry results for proteins are shown in Fig. 2. The POP group showed markedly lower expression of IGFBP5 compared to the control group (0.2949 ± 0.0045 vs. 0.2997 ± 0.0044 , $P < 0.01$). Similarly, the POP group exhibited reduced expression of collagen I (0.3138 ± 0.0062 vs. 0.3181 ± 0.0018 , $P < 0.01$) and collagen III (0.2687 ± 0.0076 vs. 0.2730 ± 0.0048 , $P < 0.05$) compared to the control group. Conversely, MMP2 expression in the POP group significantly exceeded that in the control group (0.3066 ± 0.0121 vs. 0.2806 ± 0.0093 , $P < 0.0001$). However, TIMP1 expression in the POP group was comparable to that in the control group (0.2327 ± 0.0025 vs. 0.2329 ± 0.0025 , $P = 0.865$).

Western blotting

Western blot analysis showed a obvious decrease expression of IGFBP5 (1.00 ± 0.8160 vs. 0.45 ± 0.2333 , $P < 0.01$) in the POP group. The expression levels of collagen I (1.00 ± 1.2045 vs. 0.16 ± 0.1626 , $P < 0.01$), collagen III (1.00 ± 1.5584 vs. 0.18 ± 0.0815 , $P < 0.05$) were markedly decreased in the POP group compared to the control group, as indicated by statistically significant differences. TIMP1 (1.00 ± 1.4435 vs. 0.40 ± 0.4225 , $P = 0.084$) was lower in the POP group. Conversely, western blot analysis revealed a significant increase expression of MMP2 (1.00 ± 0.8619 vs. 3.11 ± 4.1527 , $P < 0.05$) in the POP group. The results of western blot were corresponded to the immunohistochemical analysis (Fig. 3).

RT-qPCR

As observed in Fig. 4, changes in the levels of mRNA of IGFBP5, collagen I, collagen III, MMP2, and TIMP1 in the two groups were similar to the changes in their corresponding levels of protein. The mRNA level of IGFBP5 (1.00 ± 1.0543 vs. 0.43 ± 0.3842 , $p < 0.05$) in the POP group was obviously decreased compared to that in the control group. Expression of collagen I (1 ± 1.0418 vs. 0.44 ± 0.3731 , $P < 0.05$) and collagen III (1.00 ± 0.9924 vs. 0.50 ± 0.4926 , $P < 0.05$) mRNA levels in the POP group were notably reduced. While MMP2 mRNA level was significantly increased (1.00 ± 0.7221 vs. 4.13 ± 3.2018 , $P < 0.001$). The mRNA level of TIMP1, inhibitor of MMP activity, was similar in two groups (1.00 ± 0.7827 vs. 0.67 ± 0.3458 , $P = 0.093$).

Comparison of IGFBP5

To make further efforts to validate the effect of IGFBP5 in the age and the development of POP, we compared the RT-qPCR and western blot analyses of IGFBP5 in patients with different ages and degrees of prolapse. The expression levels are presented in Table 3. IGFBP5 mRNA expression in POP-Q stage III patients was obviously higher than POP-Q stage IV patients (1.00 ± 0.8741 vs. 0.25 ± 0.1576). Western blot analysis indicated that the IGFBP5 expression level in POP-Q stage III was also higher than that in POP-Q stage IV (1.00 ± 0.5891 vs. 0.31 ± 0.2070). Both the mRNA and protein results were statistically significantly different ($P < 0.05$). IGFBP5 mRNA (1.00 ± 0.8893 vs. 0.44 ± 0.3576) and protein (1.00 ± 0.6044 vs. 0.48 ± 0.2519) expression levels were obviously higher in younger POP patients (< 65 years old) when compared to older POP patients (≥ 65 years old) ($P < 0.05$). The correlation between age and IGFBP5 expression levels are presented in Fig. 5. The protein ($r = -0.7145$, $P < 0.0001$) and mRNA ($r = -0.386$, $P < 0.05$) levels were negatively correlated with age. The results of multiple regression analysis suggest that both age ($\beta = -0.643$, $P < 0.001$) and degree ($\beta = -0.346$, $P < 0.01$) of prolapse had a negative impact on IGFBP5, with age having a greater impact (Table 4).

Discussion

Within our current investigation, we analyzed the different expression levels of IGFBP5 in the lamina propria tissue among two groups. Patients with POP exhibited decreased expression levels of IGFBP5 protein and mRNA in the lamina propria tissue when compared to the control group. Previous reports have examined the expression and biological activities of IGFBP5 in various tissues, however, there has been no research on the expression of IGFBP5 or its involvement in POP. IGFBP5 is overexpressed in systemic sclerosis and idiopathic pulmonary

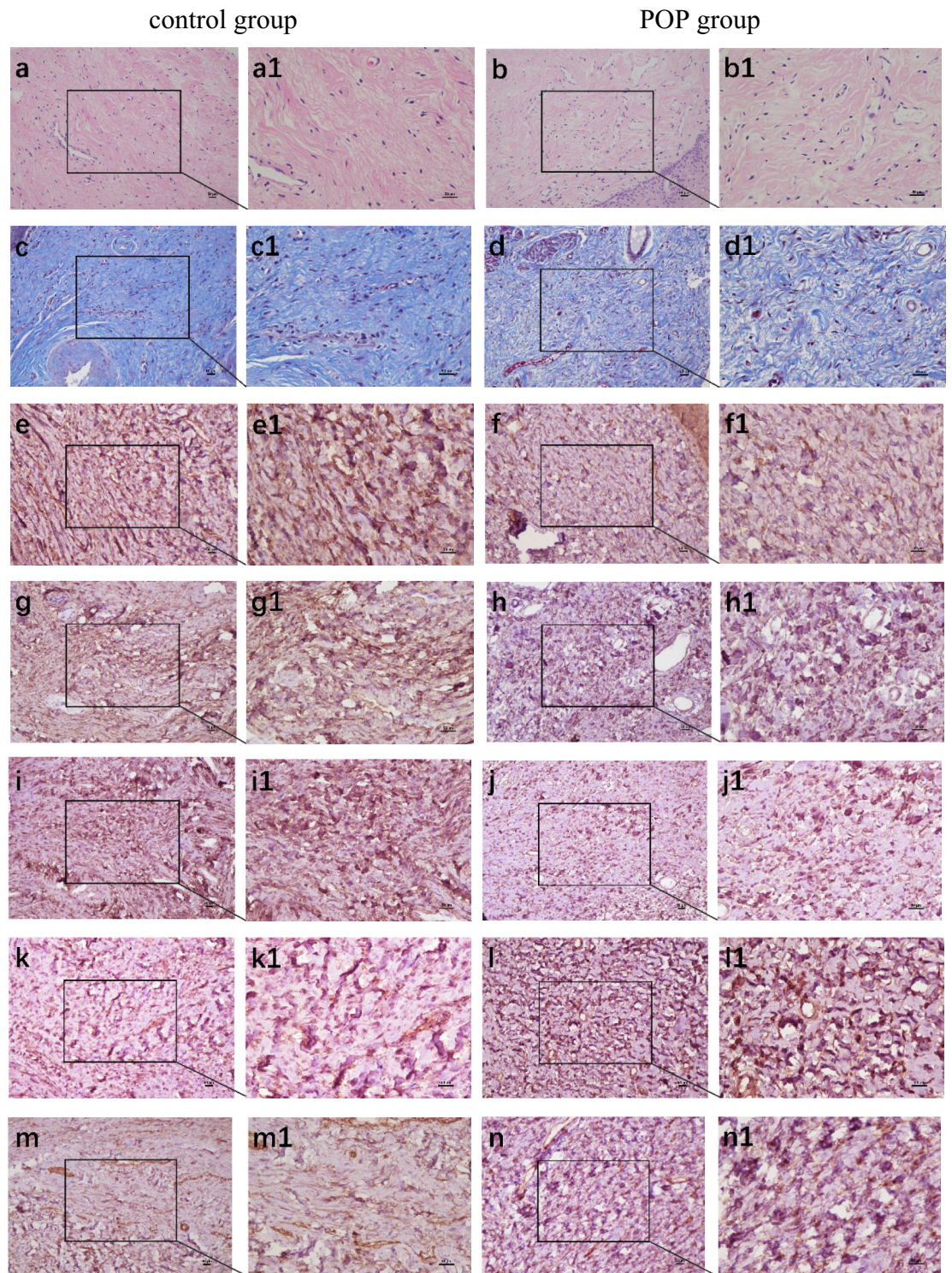


Figure 1. Light microscope observations of pictures. (a-a1) HE staining in the control group. (b-b1) HE staining in the POP group. (c-c1) Masson trichrome staining in the control group. (d-d1) Masson trichrome staining in the POP group. (e-e1) Expression of IGFBP5 in the control group. (f-f1) Expression of IGFBP5 in the POP group. (g-g1) Expression of collagen I in the control group. (h-h1) Expression of collagen I in the POP group. (i-i1) Expression of collagen III in the control group. (j-j1) Expression of collagen III in the POP group. (k-k1) Expression of MMP2 in the control group. (l-l1) Expression of MMP2 in the POP group. (m-m1) Expression of TIMP1 in the control group. (n-n1) Expression of TIMP1 in the POP group. Magnification, $\times 200$ (a-n) and $\times 400$ (a1-n1); scale bar: 50 μm .

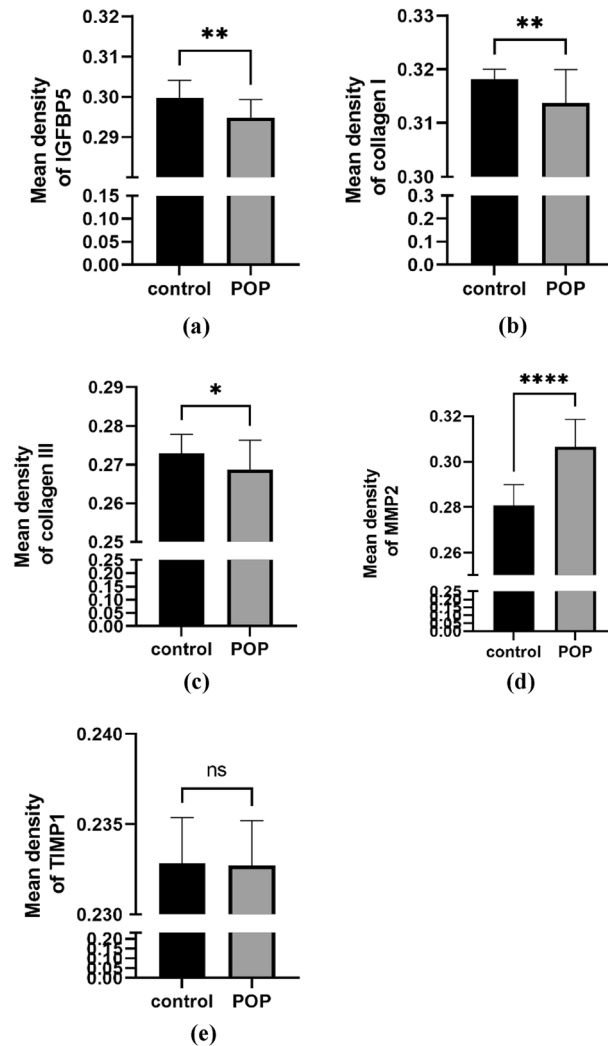


Figure 2. (a)–(e), Immunohistochemistry quantitative analysis of (a) IGFBP5, (b) collagen I, (c) collagen III, (d) MMP2, and (e) TIMP1 in the anterior vaginal wall tissues of patients in the POP group and control group. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, ns, $P > 0.05$.

fibrosis lung tissues¹⁶. IGFBP5 is downregulated in head and neck squamous cell carcinoma¹⁸ and upregulated in breast cancer and colorectal cancer^{13,19}. IGFBP5 can act as a positive or negative regulator of cellular proliferation and apoptosis under different conditions¹³. A research study found that IGFBP5 promoted the proliferation of cardiac fibroblasts, while caused apoptosis in cardiac myocytes²⁰. In primary human lung fibroblasts, the addition of exogenously recombinant IGFBP5 and the presence of endogenous IGFBP5 both stimulate the expression of the ECM and profibrotic factors⁹. While in osteosarcoma cells, endogenous and exogenous IGFBP5 exhibits contrary biological effects²¹. Li et al. noticed that IGFBP5 was observed to hinder breast cancer cells proliferation through an IGF-dependent pathway in vitro²², whereas IGFBP5 facilitates breast cancer cells proliferation in an IGF-independent manner¹³. Although IGFBP5 has a wide range of biological activities, its role in POP remains uncertain. As far as we know, no previous research has examined the correlation between declined IGFBP5 and the occurrence and progression of POP.

The results of our study also revealed changes in ECM composition-related proteins in POP patients compared patients without POP. Both collagen I and collagen III expression levels were obviously declined in patients with POP. The connective tissue in the lamina propria tissue of anterior vagina approximates the vaginal fornix (apex) was once believed to be a reflective component of the endopelvic fascia²³. Type of collagen most plentiful in interstitial connective tissue are collagen I and collagen III. Collagen I is primarily accountable for the mechanical strength of connective tissue, whereas collagen III and elastin are vital in maintaining tissue elasticity²⁴. As collagen I and collagen III are major constituents of ECM in connective tissue²⁵, maintaining an appropriate equilibrium in the synthesis and degradation of collagen are crucial for ECM. The connective tissue support structure of POP is thought to be damaged in strength rather than elasticity⁷. MMPs are important enzymes for degrading ECM. MMP2 and MMP 9 mainly decrease the accumulation of collagens²⁶, and MMP2 can degrade type I, II, and III collagen proteins²⁷. According to previous reports, TIMP1 expression decreases in

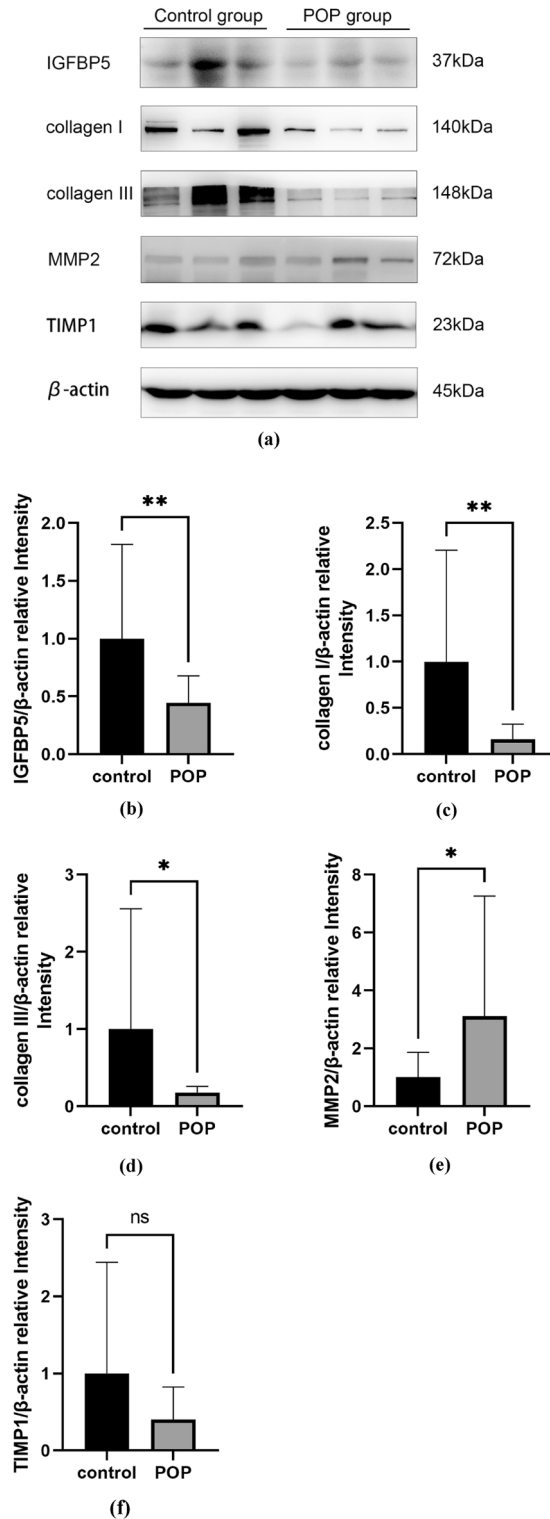


Figure 3. (a) Western blot analysis was used to detect IGFBP5, collagen I, collagen III, MMP2 and TIMP1 protein expression levels in the vaginal tissues of patients in the control and POP groups. (b) Western blot relative expression quantitative analysis of protein IGFBP5, (c) collagen I, (d) collagen III, (e) MMP2, and (f) TIMP1. The results represent the mean and standard deviation. * $P < 0.05$, *** $P < 0.001$, ns $P > 0.05$.

POP patients^{25,28}. TIMP1 inhibits various MMPs, including MMP2 and MMP9²⁹. Considering the significant changes and important roles of MMP2 and TIMP1 in collagen degradation and ECM metabolism, we assessed

	Cases	Age or POP stage	Protein expression			mRNA expression		
		<i>P</i>	Relative Protein expression	<i>t</i>	<i>P</i>	Relative mRNA expression	<i>t</i>	<i>P</i>
POP-Q stage III	22	0.294 ^a	1.00 ± 0.5891	2.778	0.01 ^c	1.00 ± 0.8741	2.077	0.048 ^c
POP-Q stage IV	6		0.31 ± 0.2070			0.25 ± 0.1576		
Age < 65	15	0.843 ^b	1.00 ± 0.6044	3.020	0.007 ^d	1.00 ± 0.8893	2.107	0.045 ^d
Age ≥ 65	13		0.48 ± 0.2519			0.44 ± 0.3576		

Table 3. Comparison of IGFBP5 expression. $P < 0.05$, statistically significant. Results are shown as mean ± standard deviation. a, comparison of age between POP-Q stage III and POP-Q stage IV. b, comparison of POP stage between age < 65 and age ≥ 65. c, comparisons between POP-Q stage III and POP-Q stage IV. d, comparisons between age < 65 and age ≥ 65.

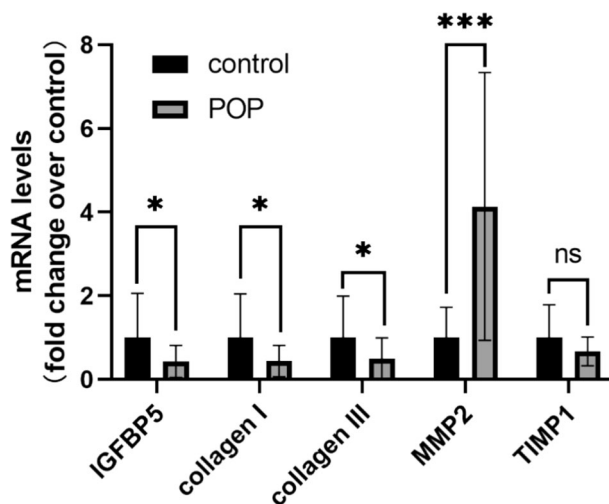


Figure 4. RT-qPCR analysis of IGFBP5, collagen I, collagen III, MMP2, and TIMP1 mRNA levels in the vaginal tissues of patients in the control and POP groups. The results represent the mean and standard deviation. * $P < 0.05$, *** $P < 0.001$, ns, $P > 0.05$.

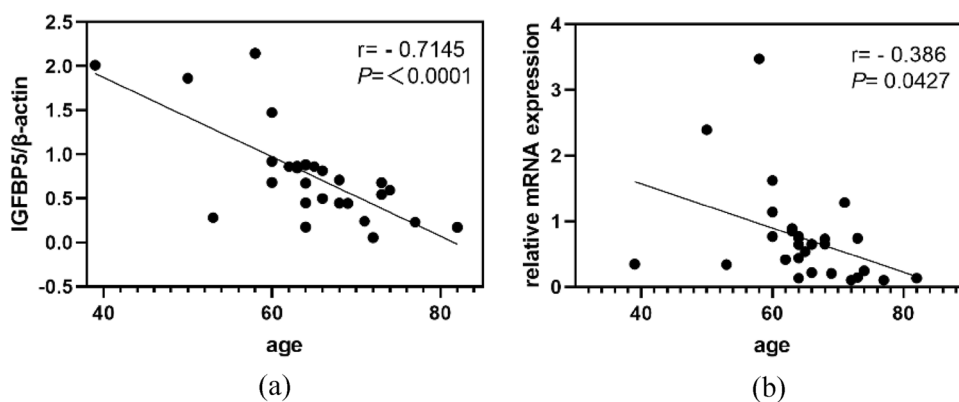


Figure 5. Correlation between IGFBP5 and age of POP, (a) relative protein expression (b) relative mRNA expression.

the levels of MMP2 and TIMP1 expression (mRNA and protein). Both mRNA and protein expression of MMP2 was obviously upregulated, while TIMP1 levels downregulated among patients with POP and compared to the patients without POP. The differences in TIMP1 were not statistically significant, possibly due to individual differences or insufficient sample size. Duan et al. found the localization of IGFBP5 within ECM in tissues, as well as its ability to bind numerous ECM proteins³⁰. In a cardiac fibroblasts study, it was observed that the introduction of recombinant IGFBP5 led to an increased ERK phosphorylation, cell proliferation, and mRNA expression of collagen III, MMP2, and MMP9²⁰. Zeeh et al. found that IGFBP-5 is likely involved in the increased expression of collagen during colitis³¹. In this study, our data are partially similar to previous data^{9,20,31}. ECM remodeling

	β	t	P
Age	- 0.643	- 5.142	<0.001
POP-Q Stage	- 0.346	- 2.767	0.01
R ²	0.625		
Adjusted R ²	0.595		
F	20.855		<0.001

Table 4. Correlation coefficient of IGFBP5 protein (n = 28). $P < 0.05$, statistically significant.

is associated with multiple factors. Previous researches have shown that IGFBP5 could enhance the production and accumulation of ECM proteins, as well as by promoting the expression of growth factors in lung and skin tissues^{9,15,32}. This study found the IGFBP5 protein is positive associate with collagen I and collagen III proteins. These findings indicate that IGFBP5 may also be related to the metabolism of the ECM by increased MMP2. We speculate that downregulation of IGFBP5 reduced the fibrotic effect of fibroblasts in POP patients. The decreased IGFBP5 could affect the synthesis and degradation of ECM related proteins (e.g., collagen I, collagen III, and MMP2), resulting in changes in protein levels. Our discovery suggest reduced expression of IGFBP5 may have an effect on the development of POP by influencing the collagen composition and metabolism of the ECM. The discovery we made establishes a foundation of IGFBP5 in the ECM of POP.

In POP patients, the younger POP patients who were under 65 years old exhibited higher levels of IGFBP5 expression compared to elder patients who were over 65 years old. Various studies have provided evidence of a decrease in IGFBP5 levels in humans due to aging or certain diseases^{10,11}. POP is an age-associated disease³³, the age of POP patients was negatively correlated with IGFBP5 expression in this study. A research proposed that reduction of IGFBP5 during proposed that the reduction of IGFBP5 during consecutive cell passages promotes the process of replicative senescence in fibroblast cells obtained from mouse embryos¹⁷. The involvement of IGFBP5 is indispensable the elevation of reactive oxygen species (ROS) and premature senescence triggered by the IL 6/sIL 6R pathway in human fibroblasts³⁴. Therefore, our results on the relationship between age and IGFBP5 indicate that the decreased expression of IGFBP5 with age may contribute to trigger the senescence and apoptosis of POP fibroblasts, resulting in a decline in collagen synthesis and a rise in degradation within the connective tissue of POP. Furthermore, the comparison of IGFBP5 between POP-Q stage III and IV showed that IGFBP5 is lower in severity of prolapse IV. The findings on the influence of age and severity provide additional evidence that IGFBP5 is effective in the advancement of POP. In summary, IGFBP5 may be a vital indicator for early diagnosis and predict the severity of POP.

Previous researches have investigated the decreased TGF- β ³⁵ and the potential mechanisms of TGF- β regulating collagen synthesis and degradation in ECM of POP patients³⁶. Both TGF- β and IGFBP5 are profibrotic factors which can increase collagen but their effects are distinct. IGFBP5 enhances fibrosis but limits scar formation to the mesenchymal compartment by preventing excessive disruption of the epithelium, while TGF- β produces uncontrolled fibrotic response both in wound repairing and idiopathic pulmonary fibrosis³⁷. IGFBP5 can interact with Bat3 to stabilize the Bat3-T β R complex and sustain TGF- β signaling in liver fibrosis³⁸. In summary, it seems that IGFBP5 and TGF- β have a synergistic effect in fibrosis and IGFBP5 has a protective effect in TGF mediated injury of fibrosis. Our results suggested that the decrease of IGFBP5 may relate to the ECM alteration of POP patients. As the possible mechanism of action for IGFBP5 in liver fibrosis³⁸, IGFBP5 may interact with Bat3 to sustain the ECM regulatory effect of TGF- β in POP. Since IGFBP5 plays an important role in the MAPK/ERK pathway in fibrotic diseases such as systemic sclerosis and idiopathic pulmonary fibrosis¹⁵, their association with the MAPK/ERK pathway may stimulate ECM production in vaginal tissue of POP. Nevertheless, it is necessary to confirm these potential mechanisms through additional experiments.

There are limitations to this study. Some unsatisfactory results may be limited by the small sample size. We only compared the different expression levels of IGFBP5 and ECM proteins in vaginal wall tissues of patients with POP and without POP but did not investigate how IGFBP5 acts on ECM and participates in the pathogenesis of POP in this study. We also cannot confirm whether the decrease in IGFBP5 causes POP or POP leads to the decrease in IGFBP5. It is important to evaluate the function of IGFBP5 through further in vitro and in vivo experiments.

Overall, the levels of IGFBP5 and proteins related to ECM were significantly reduced in vaginal tissues of POP patients. The expression levels of IGFBP5 showed a negative correlation with both age and the severity of prolapse. The findings of our research indicate that decrease in IGFBP5 expression may play a crucial part in the aging process and the occurrence of POP. IGFBP5 may be a protective factor for POP.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

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Competing interests

The authors declare no competing interests.

Additional information

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