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Original Article

Comparison of histomorphological findings of cardinal ligament in patients with and without uterine prolapse

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Abstract

Aim: This study aimed to investigate histomorphological changes in cardinal ligaments between patients with and without uterine prolapse.

Material and Methods: This study included 30 patients who underwent vaginal hysterectomy for POP-Q stage 4 uterine prolapse (Group 1) and 30 patients who underwent abdominal hysterectomy for benign reasons except uterine prolapse (Group 2) at a tertiary center hospital. Demographic data, parity, uterine weight, and histomorphological findings of cardinal ligaments were compared between the two groups.

Results: Age and parity were significantly higher unlike uterine weight was lower in Group 1. In histomorphological findings of cardinal ligaments, vessel wall thickness, peripheral nerve thickness and the number of cells in the connective tissue stroma counted in 1 mm2 area were statistically significantly higher in Group 1. While the presence of extravasated erythrocytes was greater in Group 1, no significant difference was found between the two groups in terms of inflammation.

Conclusion: It is obvious that some histomorphological changes are formed in the cardinal ligaments of patients with uterine prolapse due to pressure on the uterus. We believe that the increase in the number of extravasated erythrocytes and the thickness of the vascular wall and peripheral nerve should be supported by further studies.

Keywords: uterine prolapse; cardinal ligament; histomorphology; vessel wall; peripheral nerve; extravasated erythrocytes

INTRODUCTION

Hysterectomy is one of the most common gynaecologic proce¬dures conducted in the world (1). Hysterectomy can be performed via abdominal, vaginal laparoscopic or robotic routes depending on the previous history of pelvic surgery, uterine size, degree of descent and experience of the surgeon (2). Vaginal hysterectomy is a way of uterus removal in case of uterine prolapse (2,3). Uterine prolapse, the herniation of the uterus to or beyond the vaginal walls, is a common condition (4). The patients with uterine prolapse exist symptoms that impact exercise, sexual function and daily activities (4). Risk factors for uterine prolapse include parity, advancing age, and obesity (5).

The cardinal ligament is the primary structure that provide apical support to the uterus (6). This suspensory ligament has long been studied to better understand the development of uterine prolapse (7,8). The cardinal ligament contains vascular components such as artery and vein, nerve and soft tissue (9). The cardinal ligament can be divided based on histological characteristics. In the literature revealed variable regulation in collagen fibers and inconsistent cellular and vascular components in different regions of the cardinal ligament on microscopic examination (10). Also histomorphologic studies showed that increased some of collagen types with decreased elastin in the cardinal ligaments of patients with an uterine prolapse (11).

The aim of this study was to investigate histomorphological changes in cardinal ligaments between patients who underwent vaginal hysterectomy for stage 4 uterine prolapse and those underwent abdominal hysterectomy for benign reasons without uterine prolapse.

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MATERIAL and METHODS

Patients and study groups

This study included 30 patients who underwent vaginal hysterectomy for stage 4 uterine prolapse and 30 patients who underwent abdominal hysterectomy for benign reasons except uterine prolapse between January 2016 and July 2018 at a tertiary center. Before the study, approval was obtained from the local ethics committee (Approval no. 175).

Patients with uterine prolapse owing to known connective tissue disease, patients undergoing abdominal or laparoscopic hysterectomy despite uterine prolapse, and patients with prolapse other than stage 4 were excluded. Patients who had Pessary ring due to high anesthesia risk or for her own preference, inconvenience for lithotomy for hip prosthesis and data were not available were excluded. In addition, patients with additional pelvic pathologies such as endometriosis, myoma uteri, or malignancy as a result of final pathology were excluded from the study.

Pelvic organ prolapse was evaluated according to POP-Q staging. (12). Demographic data, parity, uterine weight, and histomorphological findings of cardinal ligaments were compared between 30 patients who underwent vaginal hysterectomy (Group 1) and 30 patients who underwent abdominal hysterectomy (Group 2).

Surgical technique and evaluation of materials

All operations were performed only specialist doctors as described by Zimmerman for vaginal hysterectomy and as described by Konushi for abdominal hysterectomy (13,14). Cardinal ligaments were bilaterally cut and ligated after the uterine artery during abdominal hysterectomy and after the uterosacral ligament during vaginal hysterectomy. Cardinal ligaments, which were surgically removed immediately after the operation, were placed in 10% formaldehyde fluid. Subsequently, all cypsimenes were followed on a fully automated tissue tracking device. Tissues were appropriately embedded in paraffin after follow-up. Then 5 µm thick sections were prepared. These preparations were deparaffinized in 70 ˚C oven for approximately 1 hour. After passing through alcohol and xylene liquids, the tissues were stained with Hematoxylin & Eosin by automatic staining device and examined. In histomorphological evaluation of the cardinal ligaments; wall thickness of the vessel which is the thickest and fits at x400 magnification, and the thickness of the peripheral nerve were measured in µm. Cell count was also measured in connective tissue area taken in µm in two dimensions in x400 magnification at the highest cellularity areas. The number of cells per 1 mm2 area was calculated. The presence of extravasated erythrocytes and inflammation in the connective tissue were examined. Inflammation was evaluated by the presence of mononuclear, polymorphonuclear leukocytes in connective tissue. All parameters evaluated with microscopic examination were photographed and shown. The examination was carried out with Olympus BX53 light microscope.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 16 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data. According to our study results, sample size of the study population was calculated to be 30 patients for each group (α= 0.05 and the power of the study= 80%). The datasets were exposed to normality tests by Kolmogorov–Smirnov method, and reported as mean ± standard deviation. Group comparisons were accomplished by using Pearson's chi-square test, Mann–Whitney U test and independent sample t-tests. The results were evaluated with a significance level of $p<0.05$.

RESULTS

In this study, demographic data and histomorphological findings of cardinal ligaments were compared between

Table 1. The comparasion between the two groups

Characteristics		Group 1 Vaginal hysterectomy n=30 Mean±SD (Min-Max)	Group 2 Abdominal hysterectomy n=30 Mean±SD (Min-Max)	p
Age		61.7±9.7 (45-79)	50.2±6 (40-63)	<0,001
Parity		6.3±1.6 (3-9)	4.5±1.7 (1-8)	<0,001
Uterine weight (g)		91.4±20.4 (63-180)	213.2±137.3 (110-687)	<0,001
Vessel wall thickness of CL(µm)		65.7±24.6 (33.02-124.93)	51±18.8 (24.95-86.61)	0,024
Peripheral nerve thickness of CL (µm)		132.8±37.8 (84.57-219.02)	100.6±28.8 (52.18-177.71)	<0,001
Connective tissue cell count of CL (1 mm^2)		3829±1952 (1430-9570)	2449±790 (1350-4500)	<0,001
Extravasated erythrocytes of CL	Yes	14/30 (46.7%)	2/30 (6.7%)	<0,001
	No	16/30 (53.3%)	28/30 (93.3%)	
Inflammation of CL	Yes	3/30 (10%)	6/30 (20%)	0,278
	No	27/30 (90%)	24/30 (80%)	

SD: standart deviation, Min: Minimum, Max: Maximum CL: cardinal ligament

30 patients who underwent vaginal hysterectomy for uterine prolapse (Group 1) and 30 patients who underwent abdominal hysterectomy without prolapse (Group 2). Age and parity were significantly higher unlike uterine weight was lower in Group 1.

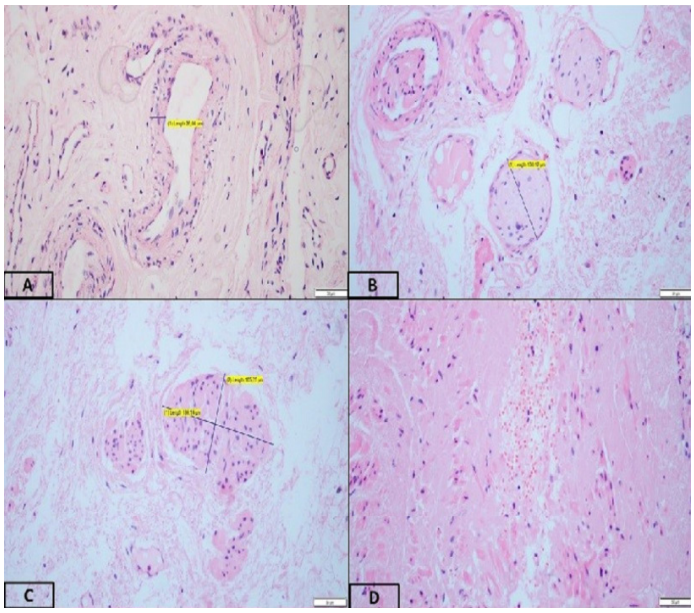

Figure 1. Cross-section of the cardinal ligament in a patient with uterine prolapse who underwent vaginal hysterectomy A- Vascular structure B- Peripheral nerve section C- Connected tissue area measured in two dimensions D- Extravasated erythrocytes and inflammatory cells in connective tissue area (x400, Hematoxylin & Eosin stained)

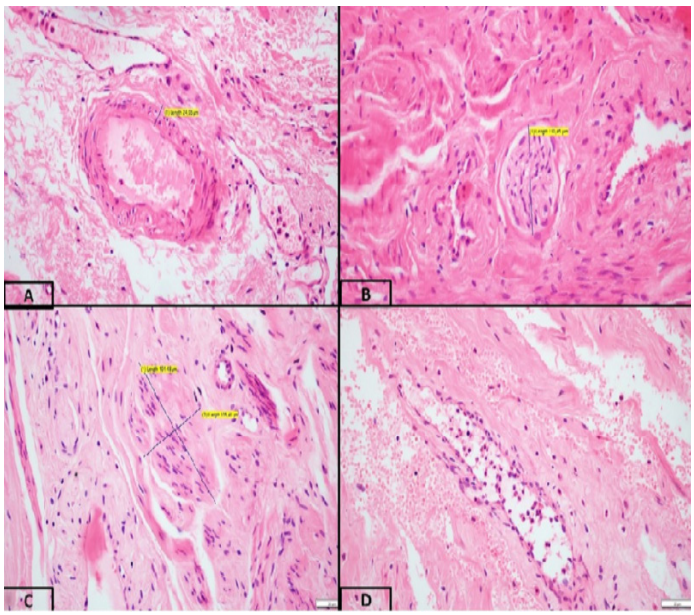

Figure 2. Cross-section of the cardinal ligament in a patient without uterine prolapse who underwent abdominal hysterectomy A- Vascular structure B- Peripheral nerve section C- Connected tissue area measured in two dimensions D- Extravasated erythrocytes and inflammatory cells in connective tissue area (x400, Hematoxylin & Eosin stained)

Evaluation of cardinal ligaments with the light microscope revealed that the vessel wall thickness was 65.7 ± 24.6, 51±18.8 µm, peripheral nerve thickness was 132.8±37.8, 100.6 ± 28.8 µm and the number of connective tissue cells counted in 1 mm2 area was 3829 ± 1952, 2449±790 in group 1 and 2, respectively. In histomorphological findings of cardinal ligaments, vessel wall thickness, peripheral nerve thickness and the number of connective tissue cells counted in 1 mm2 area were statistically significantly higher in Group 1. While the presence of extravasated erythrocytes was greater in Group 1, no significant difference was found between the two groups in terms of inflammation. The comparison of demographic data and histomorphological findings of both groups are shown in Table 1 in detail. Light microscopy images of cardinal ligaments of patients with uterine prolapse are shown in Figure 1 and images of patients without prolapse are shown in Figure 2.

DISCUSSION

In this study, histomorphologic finding of cardinal ligaments in pathology specimens of patients with and without uterine prolapse was evaluated. Vessel wall and peripheral nerve thickness and the number of connective tissue cells counted in 1 mm2 area were statistically significantly higher in patients with uterine prolapse. Also, it was found that the presence of extravasated erythrocytes was greater.

The uterus and vaginal upper 1/4 are supported by the uterosacral-cardinal ligament complex. In particular, the cardinal ligament is the most important ligament that holds the uterus in place. Uterine prolapse may occur if this ligament ruptures or becomes thinner (15). Age, parity, menopause, obesity, constipation, pelvic floor dysfunction, severe working conditions and low socioeconomic status are risk factors for uterine prolapse (5,16). In this study, advanced age and parity were considered as risk factors in accordance with the literature.

The CL is the piece of the endopelvic fascia that connects the cervix and the part of upper ¼ vagina to the pelvis. It includes vessels and peripheral nerves and having, apart from this mesentery-like nourishing role, also an important supportive role due to its collagen-rich structure (17). The increase in the number of tissue forming cells is called hyperplasia and the increase in volume is called hypertrophy (18). In patients with uterine prolapse, the load on the cardinal ligament increases due to the above-mentioned risk factors (19). Therefore, both hypertrophy and hyperplasia occur in the vessels and nerves in the ligament. In this study, in histomorphologic findings of cardinal ligaments of patients with uterine prolapse; it was detected vessel wall thickness, peripheral nerve thickness and connective tissue cell count were increased. We think this is related to the activation of compensatory mechanisms due to increased load and damage in the ligament.

In a previous study, the relationship between pelvic organ prolapse and connective tissue structure was evaluated.

Tenascin-X expression in uterosacral ligament was investigated in patients with and without prolapse. In the study it was found that Tenascin-X was expressed in 94% of pelvic organ prolapse cases and 91% of controls. While there was no significant difference between the two groups, Tenascin-X was found to be more commonly expressed in patients with POP-Q stage 4 uterine prolapse. (20). Two studies have suggested that hypoxia can cause pelvic organ prolapse with apoptosis. Both studies evaluated Hypoxia-inducible factor-1α and found that hypoxia-inducible factor-1α expression was higher in patients with uterine prolapse. (21,22). Similarly, in another study, heparanase expression was found more common in patients with uterine prolapse. (23). In contrast, another study reported that patients with pelvic organ prolapse had lower levels of ADAMTS-2, collagen type-1, TIMP-3 and papilin in the cardinal ligament compared with no pelvic organ prolapse (24). In all of these studies, especially connective tissue stroma was evaluated. However, other elements of connective tissue such as vessel wall or peripheral nerve thickness have not been evaluated. In this study, similar to other studies, the number of cells in connective tissue stroma was increased in patients with prolapse compared to those without prolapse. The vessel wall and peripheral nerve thickness were also increased in patients with uterine prolapse. The presence of extravasated erythrocytes was greater in patients with uterine prolapse and no significant difference was found between the two groups in terms of inflammation. We conclude that extravasated erythrocytes are more common in patients with prolapse and are exposed to greater trauma and stress due to the presence of the uterus outside. Also surgical technique or bleeding during the operation in patients with prolapse could be responsible for extravasated erythrocytes.

The limitation of this study is that it is a retrospective study, which could be open to selection bias. The same, it lacks data on smoking status, diabetes mellitus and body mass index. Evaluation of a small number of patients is yet another limitation. The strength of the study is the evaluation of not only connective tissue cells but also vascular wall and peripheral nerve thickness and extravasated erythrocytes in the cardinal ligaments of patients with and without uterine prolapsus. This study is the first to evaluate vessel wall thickness, peripheral nerve thickness, connective tissue cell count in the cardinal ligament of patients with and without prolapse.

CONCLUSION

In conclusion, it is obvious that some histomorphological changes occur in the cardinal ligaments of patients with uterine prolapse. These changes are the increase in vessel wall and peripheral nerve thickness, the number of cells in the connective tissue stroma and the amount of extravasated erythrocytes. We believe that the increase in the number of extravasated erythrocytes and the thickness of the vascular wall and peripheral nerve should be supported by further studies.

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