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Prolidase Enzyme Activity in Endometrial Polyps and Its Relationship with Oxidative Stress

Endometriyal Poliplerde Prolidaz Enzim Aktivitesi ve Oksidatif Stres ile İlişkisi

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Öz

Endometrial polip (EP) kadın hastalıkları ve doğum kliniği pratiğinde sıklıkla karşılaşılan bir durumdur. Biz çalışmamızda endometrial polip tanısı olan hastaların prolidaz enzim aktivitesi ve oksidatif stres (OS) ile ilişkisini ortaya çıkarmayı amaçladık. Araştırmaya dahil edilme kriterlerini karşılayan histopatolojik olarak EP tanısı konan 35 hasta ile endometrial örnekleme sonucunda patoloji tespit edilmeyen 35 anormal uterin kanaması (AUK) olan kontrol grubu hastası dahil edilmiştir. EP ve kontrol grupları arasında TAS, TOS, OSI, prolidaz ve endometrium kalınlığı değerleri karşılaştırıldığında EP grubunda, kontrol grubuna göre TOS, OSI, prolidaz ve endometrial kalınlık değerleri istatistiksel olarak anlamlı düzeyde yüksek bulunmuştur. Çalışmamız, daha geniş hasta grupları ile yapılan çalışmalarla desteklendiğinde patogenezin daha ayrıntılı anlaşılması ve klinik olarak hasta takibinde yararlı bir belirteç olabileceği görüşündeyiz.

Anahtar Kelimeler: Endometrial Polip, Oksidatif Stres, Prolidaz Enzim Aktivitesi

Abstract

Endometrial polyp (EP) is a condition that is often encountered in obstetrics and gynecology clinic practices. In our study, we aimed to reveal the relationship between prolidase enzyme activity (PEA) and Oxidative Stress (OS) in patients with endometrial polyps. Thirty-five patients who were histopathologically diagnosed with EP and 35 patients with abnormal uterine bleeding (AUB) without pathology as a result of endometrial sampling were included in the control group. Serum TOS, OSI, tissue PEA, and endometrial thickness values were found to be statistically significantly higher in the EP group compared to the control group. We believe that our study, when supported by studies with larger patient groups, may be a useful marker for a more detailed understanding of the pathogenesis and clinical follow-up of patients.

Keywords: Endometrial Polyp, Oxidative Stress, Prolidase Enzyme Activity

Introduction

Endometrial polyps (EPs) are benign growths that extend from the endometrial surface into the cavity. They arise due to hyperplasia of the endometrial gland and stroma surrounding a vascular structure (1). Its incidence is estimated to be about 8% in the general population, and between 10% and 30% in females with abnormal uterine bleeding (AUB) (2,3). Advanced age and tamoxifen use are the most important known risk factors (4). From this background, endometrial cancer may develop (5). In addition to malignancy potential, in various sources, it has been reported that it can regress spontaneously at different rates (27.0-57.1%) (6). The EP diagnosis is made after histopathological evaluation with endometrial sampling.

The pathophysiology of EP is indeed multifactorial and complex. Increased expression of Bcl-2, a protein that regulates cell death, and estrogen receptors, which mediate the effects of estrogen, have been implicated in the development of EP. Estrogen-dependent causes relate to the actions of estrogen, a hormone associated with the female reproductive system. These conditions can fall under hypoestrogenism or hyperestrogenism, or any sensitivity to the presence of estrogen in the body. Estrogen-dependent cancers, like breast cancer, ovarian cancer and endometrial cancer, rely on estrogen to develop and grow. On the other hand, estrogen-independent causes do not rely on the presence of estrogen. These could be due to mutations that confer estrogen-independent activity to estrogen receptors, causing changes in gene expression. Some cancers can develop resistance to endocrine therapy, which is used to block the action of estrogen in estrogen receptor-positive cancers. However, the exact mechanisms and causes behind EP, particularly in different periods such as premenopausal and postmenopausal stages, are not yet fully understood. Research is ongoing to further understand these mechanisms and improve treatment strategies for EP (6).

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Oxidative stress (OS) is indeed a state of imbalance between the production and elimination of reactive oxygen species (ROS) in the body. When the production of ROS increases or the body's ability to eliminate them decreases, it leads to an accumulation of these reactive species, disrupting the balance and causing OS.

OS is a key factor in causing tissue or molecular damage in cells. It's been implicated in a variety of health conditions, including neurodegenerative diseases, cancer, and cardiovascular diseases (7).

In the context of endometriosis, recent studies have highlighted the role of OS. It's defined as an imbalance between ROS and antioxidants, which may be implicated in the pathophysiology of EP, causing a general inflammatory response in the peritoneal cavity. Local inflammation and increased levels of ROS contribute to the acquisition of a proliferative phenotype and proangiogenic features that are crucial to endometriotic lesion development (8).

However, more research is needed to fully understand the complex mechanisms by which OS contributes to diseases like EP and to develop effective therapeutic strategies (7). In the literature, OS status in EPs was evaluated using serum catalase, xanthine oxidase, and malondialdehyde, and as a result, data showing that OS may have a role in the pathophysiology were obtained (9).

Prolidase is a type of metalloproteinase that is found in abundance throughout the body. It plays crucial roles in various biological processes, including cell proliferation, collagen metabolism, and matrix remodeling. There is a significant correlation between prolidase activity and increased collagen turnover (10). It's hypothesized that an increase in collagen turnover may play a role in the pathophysiology of polyps, and this could potentially be determined by prolidase activity. However, to date, there have been no studies that have explored the role of the enzyme prolidase in the pathophysiology of EPs (11).

In our study, we aimed to compare the group of patients diagnosed with EP and the control group and to reveal the relationship of EPs with prolidase enzyme activity (PEA) and OS.

Material and Method

This study is a prospective case-control study that was conducted between December 1, 2021, and March 1, 2022, at the Department of Obstetrics and Gynecology, Muğla Sıtkı Koçman University Faculty of Medicine (MSKU). The study received approval from the MSKU Faculty of Medicine Clinical Research Ethics Committee on January 6, 2021 (Decision no: 1/II) and was supported by the MSKU Scientific Research Projects Coordination Unit (Project No: 22/136/02/3/4). All patients were informed about the study and their written informed consent was obtained.

The study involved patients who met the inclusion criteria and were histopathologically diagnosed with EPs. The control group was composed of patients with abnormal uterine bleeding (AUB) who, following endometrial sampling, were found to have no pathological conditions.

Exclusion Criteria

The study did not include patients who had abnormal pap smear results, an adnexal mass, or a diagnosis of malignancy. Additionally, individuals with other pathological conditions that could lead to oxidative stress, such as pulmonary disease, pulmonary hypertension, inadequate cardiac function, renal and hepatic dysfunction, chronic ischemic disease, and systemic inflammatory disease were also excluded. Smokers, alcohol users, substance abusers, and those who take antioxidant vitamins and lipid-lowering drugs were not part of the study either.

Data Collection Tools

Within the scope of the study, data collection forms and biochemical analysis methods (laboratory analysis of blood and tissue samples) were used as data collection tools.

Laboratory Analysis of Blood and Tissue Samples

The polyp tissue that was surgically removed was first rinsed with 0.09% NaCl before being placed into Eppendorf tubes. These tubes were then stored in a deep freezer (Thermo Scientific, -80°C) located in the Biochemistry Department Research Laboratory at Muğla Training and Research Hospital. The samples remained there until the day of the study.

On the day of the study, each tissue sample was first weighed to ensure it was approximately 100±10 mg. The samples were then homogenized in a cold phosphate buffer (PBS; pH: 7.4; 50 mM) at a ratio of 1/10 using a homogenizer (IKA T10 Ultra-Turrax 10) operating at 20,000 rpm. Following this, the homogenate of the polyp tissue was centrifuged at 10000 xg for 5 minutes with Hettich Mikro 200 centrifuge (Andreas Hettich Co., Tuttlingen, Germany) and a temperature of +4°C for a duration of 5 minutes.

Approximately 6 cc of venous blood was taken from all females in the study and control groups into tubes containing separators. The blood was centrifuged at 2000 xg for 10 minutes at +4 and serum samples were taken. The samples were stored at -80°C. Prolidase with the supernatant obtained from the tissue homogenate, total antioxidant status (TAS) obtained from the serum, and total oxidant status (TOS) tests were studied from blood.

Prolidase Activity

PEA was quantified using the modified Chinard method and a photometric method with a commercial kit (Rel Assay Diagnostics, Türkiye, Catalog no: RL0025). The underlying principle of the method is that proline, a component of the glycine-proline substrate produced by the prolidase enzyme, forms a colored compound with ninhydrin under the influence of heat in an acidic environment. The color intensity is proportional to the proline concentration. The absorbance of the resulting proline was measured at 515 nm, and the enzyme activity was expressed as U/L creation (10).

Total Antioxidant Status (TAS)

TAS levels were studied colorimetrically (Rel Assay Diagnostics, Türkiye, Catalog no: RL0017) by taking 18 µL of supernatant (12). The assay was conducted using an ELISA plate, and the results were determined by performing two readings at a wavelength of 660 nm with an ELISA reader, with 5-minute intervals between each reading. The standard curve was established using the company's calibrator. This test demonstrated a repeatability value of %CV<10% and the reading range was between 0.1-3.5 mmol TroloxEq/L.

Total Oxidant Status (TOS)

TOS levels were studied colorimetrically (Rel Assay Diagnostics, Türkiye, Catalog no: RL0024) by taking 45 µL of supernatant (13). The assay was carried out using an ELISA plate, and the results were determined by taking two readings with an ELISA reader at a wavelength of 530 nm, with a 5-minute interval between the readings. The standard curve was created using the company's calibrator (Catalog number RL0024). This test had a repeatability value of %CV<10% and the reading range was between 0.2-80 µmol H_2O_2Eq/L.

Calculation of Oxidative Stress Index (OSI)

The OSI is the ratio of the TOS value in µmol H_2O_2Eq/L to the TAS value in mmol TroloxEq/L (14).

Data Analysis

The research data was analyzed using the SPSS 21.0 statistical software. The normal distribution of continuous variables was assessed using both visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics for the study included mean and standard deviation for data that followed a normal distribution, and median, minimum, and maximum for data that did not follow a normal distribution. The Student t-test was employed to compare continuous variables with parametric properties between independent groups, while the Mann-Whitney U-Test was used for comparing continuous variables without parametric properties between independent groups. A p-value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics of the cases in the patient and control groups are compared in Table 1. When the table is examined, no statistically significant relationship was found between the patient and control groups in terms of age, weight, BMI, gravidity, parity, and menopausal status (p<0.005).

Table 1. Comparison of demographic characteristics of cases in the control and patient groups.

		Group				
		Control (n:67)		Patient (n=35)		p
		n	Median (min-max)	n	Median (min-max)	
Age (years)		35	44.0 (27.0-72.0)	35	45.0 (31.0-66.0)	0.986
Weight (kg)		35	72.0 (58.0-110.0)	35	74.0 (59.0-97.0)	0.293
BMI (kg/m^2)		35	28.0 (22.0-39.0)	35	29.0 (24.0-82.0)	0.512
Gravidity (n)		35	2.0 (0.0-5.0)	35	2.0 (0.0-5.0)	0.333
Parity (n)		35	2.0 (0.0-3.0)	35	2.0 (0.0-4.0)	0.240
Menopause	**No**	27	77.1	27	77.1	1.000
	Yes	8	22.9	8	22.9	

TAS, TOS, OSI measurement values in serum samples of the cases, PEA measurement values in tissue samples and endometrial thickness measurement results were evaluated. In this assessment mean TAS, mean TOS, mean OSI, mean tissue PEA, and mean endometrial thickness for all study group was found to be 1.4±0.2 mmol/L, 8.4±8.5 µmol/L, 0.6±0.7 AU, 24.8±13.8 U/L and 6.4±11.3 mm, respectively.

The TAS, TOS, OSI values measured in serum samples, PEA values measured in tissue samples, and endometrial thickness measurements of both the patient and control groups were compared (Table 2). Upon examining the table, it was found that the serum TOS, OSI, tissue PEA, and endometrial thickness values were statistically significantly higher in the patient group compared to the control group (p:0.027, p:0.043, p:0.046, p:0.000, respectively).

Table 2. The comparison of serum TAS, TOS, OSI, tissue prolidase enzyme activity and endometrial thickness values between the patient and control groups.

	Group				p
	Control (n:67)		Patient (n=35)		
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
TAS (mmol/L)	1.4±0.2	1.4 (1.0-1.8)	1.4±0.2	1.4 (0.4-1.9)	0.747*
TOS (µmol/L)	7.2±9.1	3.5 (1.2-39.1)	9.6±7.8	8.0 (2.2-29.4)	**0.027***
OSI (AU)	0.5±0.6	0.3 (0.1-2.5)	0.8±0.8	0.5 (0.2-4.2)	**0.043***
Prolidase (U/L)	21.7±13.5	23.6 (1.7-64.1)	27.9±13.5	26.6 (5.8-62.3)	**0.046***
Endometrial thickness	2.7±11.4	0.0 (0.0-67.0)	10.2±10.0	8.0 (0.0-60.0)	**0.000***

Discussion

EPs are tumor-like formations covered by epithelial cells, primarily composed of endometrial glands and stroma, with a robust vascular network. These are primarily a result of excessive cellular proliferation. Several molecular mechanisms have been proposed to play a role in the development of EPs. These include the overexpression of endometrial aromatase, monoclonal endometrial hyperplasia, and gene mutations (14).

Another theory is that it was formed as a result of an irregularity in the mechanism of apoptosis (15). In a study conducted by Erdemoglu et al., EP formation was found to be associated with inflammation (16). In the literature, the OS status in EPs was assessed using serum catalase, xanthine oxidase, and malondialdehyde. The results obtained provided data suggesting that OS may play a role in the pathophysiology of EPs. In addition to OS markers, there is no information in the literature about the relationship between endometrial activity and the prolidase enzyme, which plays crucial roles in cell proliferation, collagen metabolism, and matrix remodeling. Our study elucidates the association of EPs with OS and PEA.

In our research, the average age of patients with endometrial polyps was determined to be 45.4±8.8 years (range: 27-72 years). When compared with the control group, there was no statistically significant difference in the average age values. These findings are consistent with the literature. In a study conducted by Demirtaş et al., no significant difference was observed in terms of menopause status when comparing patient groups with and without endometrial polyps (17). Özgen et al. also reported that no significant difference was found in menopause status when the EP cases and the control group in their study were compared (17). In our study, when the cases and control groups were compared in terms of menopause status in accordance with the literature, no statistically significant difference was found.

In the study of Nappi et al., multivariate analyses were used and no relationship was demonstrated between BMI, obesity, menopausal status and EP (4). Similarly, in the study of Çınar et al., no significant difference was found between the EP and control groups in terms of BMI (9). In our study, the groups were compared in terms of BMI in accordance with the literature; the mean BMI was found to be 28.9±4.4 kg/m^2 in cases with EP and 30.5±9.4 kg/m^2 in the control group, and it was found that this difference was not statistically significant.

Inflammation and OS pathways are indeed closely related. In the study by Çınar et al., they examined the relationship between some markers that play a role in OS and EPs. However, we couldn't find the specific details of Çınar et al.'s the study in current resources (8). In patients suspected of having EPs based on transvaginal ultrasound (TV-USG), blood samples were collected and levels of catalase, xanthine oxidase, and malondialdehyde were assessed. The results revealed higher levels of catalase, xanthine oxidase, and malondialdehyde in patients with EPs compared to the control group. These findings suggest a relationship between OS and EPs.

However, a study by Özgen et al. that compared TAS, TOS, and OSI levels between EP cases and a control group found no statistically significant difference between the groups (17). This indicates that the relationship between OS and EPs might be complex further investigation (18).

In a study by Nayki et al., the levels of superoxide dismutase, catalase, glutathione reductase, TAS and TOS were evaluated in all cases with endometrial biopsy due to AUB. No significant difference was found in terms of TOS and TAS levels between the patients divided into groups according to histopathologies (18). In both studies, cases with AUB that had histopathological differences were evaluated. It was suggested that the existing uterine bleeding could lead to an increase in oxidative products due to the inflammation it causes. In our study, we compared the OSI, TOS, and TAS levels of patients with EP and the control group. We found that the TOS and OSI levels were statistically significantly higher in patients with EP. Our study supports the data from Çınar et al., demonstrating a correlation between the presence of EP and OS (19).

In addition to OS markers, literature information on the relationship between endometrial activity and the prolidase enzyme, which has important functions such as cell proliferation, collagen metabolism and

matrix remodeling, could not be reached. However, studies are showing the relationship between PEA and other gynecological diseases. It has been shown that prolidase activity is increased in epithelial ovarian cancer (20). In a study, it was found that serum prolidase activity was high in patients with early pregnancy loss, and placental prolidase activity was found to be low due to possible placental use. With this study, it was concluded that low prolidase activity in the placenta may be an etiopathological factor in women with early pregnancy loss (21). Hilali et al. found that serum prolidase activity, OSI and TOS were significantly higher in patients with polycystic ovary syndrome (PCOS) compared to the control group. It was found that prolidase activity was positively correlated with TOS, follicle count and prolactin levels in patients (22). In our study, it was found that the PEA was significantly increased in patients with EP compared to the level of the prolidase enzyme in the control group.

Conclusion

This is the first study in the literature to examine EP and prolidase activity, and if these results are supported by studies with larger patient groups, it can be a useful marker for a more detailed understanding of EP pathogenesis.

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Conflict of interest statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Committee Approval: Ethical approval was obtained from the MSKU Faculty of Medicine Clinical Research Ethics Committee, Türkiye (Date: 06/01/2021, Decision no: 1/II).

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