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Investigation of Galectin-3 Levels of Endometriosis Patients According to Stages

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ABSTRACT

Objective: Endometriosis is a gynecological disease associated with chronic pelvic inflammation, pain, and infertility. Galectin-3 (Gal-3) is a protein that can bind to β-galactosides, which plays an important role in different biological processes according to the stages of the disease in patients with endometriosis. This study aimed to elucidate the importance of Gal-3 in endometriosis, to reveal its potential power as a non-invasive diagnostic biomarker in disease etiopathogenesis.

Materials and Methods: The serum concentration of Gal-3 and cancer antigen 125 (CA-125) from whole blood were measured using enzyme-linked immuno sorbent assay (ELISA) and an auto-analyzer, respectively. Gal-3 expression was determined by quantitative real-time polymerase chain reaction (qRT-PCR) from peripheral blood.

Results: We found significant differences for Gal-3 expression levels between the endometriosis and control groups ($p < 0.05$). Gal-3 levels in the serum of women with endometriosis are also remarkably increased compared with the control group.

Conclusion: Galectin-3 can play critical functions in the development and progression of endometriosis, so, further studies are needed in this area.

Keywords: Galectin-3, endometriosis , biomarkers, qRT-PCR

INTRODUCTION

Endometriosis is defined by the presence of endometrial glands and stroma outside the uterus, which is associated with pelvic pain, inflammation, and infertility in young women (1,2). The multifactorial etiopathogenesis of endometriosis includes environmental, genetic, epigenetic, endocrine or immune factors (3-6). Although the pathophysiology of the disease is not fully understood, there is increasing evidence of chronic dysregulation of inflammatory and vascular signaling in endometriosis (7). Patients often complain of dysmenorrhea, dyspareunia, pelvic pain and infertility, all of which lead to a reduced quality of life (8). Endometriosis is considered histologically a benign disease. But, development is similar to malignancy with regard to infiltration and attachment properties to other tissues (9, 10). It has been stated that endometriosis is mostly associated with ovarian cancer among all neoplasms. Many risk factors such as early menarche, short menstrual cycle duration and low parity are common for ovarian

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cancer and endometriosis (11). Diagnosis of endometriosis is delayed due to the complexity of the pathogenesis and the variety of symptoms (12). Laparoscopy is still the only reliable "gold standard" for diagnosing endometriosis. Laparoscopy is a surgical procedure with potential risks, and can be uncomfortable and sometimes even painful for the patient. From a clinical point of view, the development of a non-invasive test is important in the early diagnosis of endometriosis. Many molecules included in the pathogenesis of endometriosis have been examined as potential biomarkers. Nevertheless, they are not in the desired sensitivity and originality (13, 14). One of the most researched sources of biomarkers is peripheral blood (15). Peripheral blood is an important source of biomarkers because it is readily available and minimally invasive (16). Much research has been done on blood-based biomarkers for endometriosis (17). In recent years, lectins have become an important topic for reproductive immunology, inflammation and endometriosis (18, 19). Galectins (Gal) are glycan-binding proteins and are found in almost all organisms (20). Galectin-3 (Gal-3) has different roles in many biological processes; cell embryogenesis, adhesion, differentiation and proliferation. Although endometriosis is primarily an endometrioid type ovarian cancer, it can turn into many gynecological cancers (21). Gal-3 is predominantly found in the cytoplasm and secreted into biological fluids including serum and urine (22). Noel et al. reported that Gal-3 is increased in peritoneal endometriosis, different lesions, and eutopic endometrium. These data suggest that Gal-3 can promote the development or progression of endometriosis (18).

The high expression of Gal-3 in endometriosis suggests that these molecules could be potential diagnostic biomarkers for endometriosis and/or targets of new therapeutic approaches (23).

This study aimed to report Gal-3 expression levels in the stages of endometriosis patients, and to reveal their potential power as a non-invasive diagnostic biomarker.

MATERIALS AND METHODS

Study Design and Patients

In this study, the patient group consisted of 50 female patients who were pre-diagnosed with endometriosis (based on radiological and biopsy and histopathological results) in the Sivas Cumhuriyet University, Faculty of Medicine, Research and Practice Hospital, Gynecology and Obstetrics Clinic.

Seventy healthy volunteers without any pre-diagnosis were included in this study. The patients between the ages of 15-45 were selected and included in the study. Before the study, approval was obtained from the Ethics Committee of Cumhuriyet University, Faculty of Medicine (2019-10/06).

Approximately 5 ml of venous blood from each right or left forearm of 50 women diagnosed with endometriosis and 70 healthy women were collected into dry tubes. Patients that may affect cancer antigen 125 (CA-125) levels and mimic endometriosis as a history of rheumatic disease, chronic inflammatory disease or an autoimmune disease were not included in the study.

The study was carried out on the whole blood and EDTA containing peripheral blood serum samples taken from the patients. The whole blood samples were centrifuged at 3000 rpm for 10 minutes. Serum samples were stored at -80°C until the biochemical analysis. The remainder of the whole blood samples were separated to prepare total RNA and cDNA for Gal-3 expression by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) analysis.

Determination of CA-125 Levels

CA-125 levels, one of the best known and most widely used biomarkers for endometriosis, were measured using autoanalyzer (Roche Cobas e802, USA).

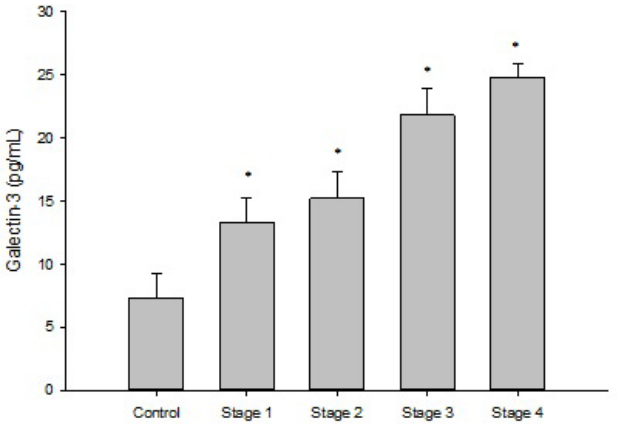

Figure 1. Results of Gal-3 levels in serum by ELISA. *: p<0.05, compared to the control.

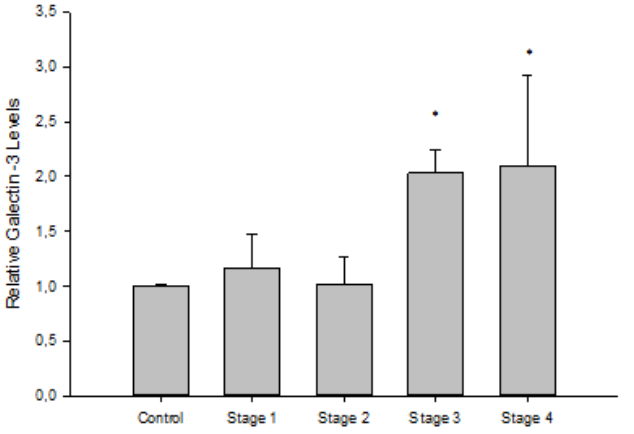

Figure 2. Results of qRT-PCR analysis Gal-3 levels between endometriosis and control group. *: p<0.05, compared to the control.

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qRT-PCR Analysis

The total RNA was extracted from whole blood collected by using the RNA Isolation Kit (GeneAll, Cat no:106-101, Korea) according to the manufacturer's recommendations. cDNA synthesis using a HyperScript cDNA synthesis kit (GeneAll Cat no: 601-710, Korea), according to manufacturer's protocols. Reaction mixtures were incubated at 25°C, 10 min; 55°C, 60 min; and 85°C, 5 min. cDNAs were measured using a qubit ssDNA Assay Kit (Molecular probes, Life Technologies, Cat No: Q10212, United States).

RT-qPCR was performed using Realamp sybr green master mix with high-ROX dye (Cat no:801-051, Korea), according to manufacturer's protocols. About 20µL PCR reaction included 4µL RT product, 1 µL (10pm) forward primer, 1 µL (10pm) reverse primer, 1µL ROX, 3 µL sterile water, and 10 µL (2X) SYBR master mix. Forward and reversed primers of Gal-3 are shown in Table 1. Gal-3 expression levels were normalized to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same sample. The PCR reaction mixtures were incubated in at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 40 s. Relative increases in mRNA expression were processed using the $2^{-\Delta\Delta CT}$ method (24).

Determination of Gal-3 Levels by ELISA Assay

Serum Gal-3 levels were analyzed by an ELISA assay, according to the manufacturer's recommendations (Elabscience, Cat No: E-EL-H1470,USA).

Statistical Analysis

The data of this study were analyzed with the statistical program SPSS-22 (SPSS INC., Chicago, IL, USA). When α= 0.05, β= 0.10, (1- β)= 0.90 (R: Sample Allection Ratio:1.7) values were taken for this study, it was decided to include 50 patients and 70 non-patients in the study. The power of the test was determined as p=0.90064. The dependent variable of the study is endometriosis disease. Its main independent variable is Gal-3 level. Data indicated by measurement are presented descriptively with mean and standard deviation (minimum - maximum), data indicated by counting, number and percentage distribution. Student t test was used to compare the mean age of both groups, and the chi-square test was used for the analysis of nominal values. When the parametric test assumptions were fulfilled (Kolmogorof Smirnov), the significance test of the difference between the two means in independent groups was used. $p < 0.05$ was considered as statistically significant.

Table 1. Forward and Reverse Primers of Gal-3 expression.

Primers	5′ → 3′
Galectin-3	AGCCAACGAGCGGAAAATG (F)
	GCACTTGGCTGTCCAGAAGA (R)
GAPDH	GTCAAGGCTGAGAACGGGAA (F)
	AAATGAGCCCCAGCCTTCTC (R)

F: Forward; R: Reverse; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

RESULTS

The demographic baseline characteristics of the patient's examined groups are given in Table 2. The only variable between the patient and control groups was the surgical stages. The endometriosis with patients who underwent laparoscopy and laparotomy were divided into endometriosis stages, that are shown in Table 3. The patients were surgically divided into stages considering the Revised American Fertility Society criteria. According to the demographic data, the patients' ages, family histories and the presence of chronic pain had no statistically significant difference. CA-125 levels were found to be statistically significant in the endometriosis and control groups ($p < 0.001$). The presence of a history of infertility, whether there was a history of pregnancy in the past, menstrual pain, and pain during sexual intercourse were found to be statistically significant between the patients and controls (Table 2). As shown in Figure 1, the Gal-3 values of the endometriosis group are approximately 13.26 ± 1.96 pg / L for stage 1, 15.18±2.08 pg / L for stage 2, 21.79±2.13 pg / L for stage 3 and stage 4 as 24.79±1.07 pg / L, while the Gal-3 value of the control group was found to be 7.29±1.98 pg / L ($p < 0.05$, for all).

The results of the qRT-PCR analysis, no significant difference was found for Gal-3 levels between endometriosis and control group in stage 1 and 2. We found significant differences in Gal-3 expression levels among endometriosis with stages 3 and 4, and the control group ($p < 0.05$, for both) (Figure 2).

DISCUSSION

Endometriosis is an important gynecological disease associated with chronic pain and infertility, affecting the quality of life. It is known that many factors such as environmental, genetic, epigenetic, endocrine or immune factors are involved in the etiopathogenesis of endometriosis (3, 5). Although it is a comprehensive field of study, there are no reliable, specific biomarkers of endometriosis (25). Therefore, a novel biomarker with high specificity and sensitivity is needed for the diagnosis of endometriosis (26). Early diagnosis is significant for the early treatment of endometriosis and helps improve quality of life and preserve fertility (25).

Gals are a family of galactoside binding proteins that plays a role in many physiological and pathological processes, such as regulation of the immune system, cell growth, and angiogenesis (14, 23). High expression levels of Gals in patients with endometriosis and associated inflammation suggest that these molecules can be used as potential diagnostic biomarkers (23). Accumulating evidence from the literature shows that Gal-3 could be used as a diagnostic or prognostic biomarker for heart disease, kidney disease, and cancer (22, 27, 28). In recent years, lectins have become important in research on the immunity of the female reproductive system, pregnancy and infertility (5,14).

Table 2. Clinical characteristics in patient and control groups.

		Patient	Control	p-value
Ages[α]	(years, mean±SD)	38.36±9.667	36.41±13.41	0.383
		n (%)	**n (%)**	**p-value**
Family history[β]	-	40 (80)	64 (91.4)	0.101
	First degree relative	9 (18)	4 (5.71)	
	Distant relative	1(2)	2 (2.89)	
Infertility[β]	+	12 (24)	7 (10)	0.038*
	-	38 (76)	63 (90)	
Past pregnancy history[β]	+	12 (24)	5 (7.14)	0.050*
	-	0 (0.0)	2 (2.85)	
Painful menstruation[β]	+	38 (76)	34 (48.6)	0.002*
	-	12 (24)	36 (51.4)	
Dyspareunia[β]	+	23 (46)	47 (67.1)	0.038*
	-	3 (6)	3 (4.4)	
Chronic pelvic pain[β]	+	24 (48)	26 (37.2)	0.234
	-	26 (52)	44 (62.8)	
CA-125 levels[β]	Low	19 (38)	54 (77.1)	0.001*
	High	31 (62)	16 (22.9)	

+: Yes; -: No; n: number; *: $p < 0.05$, compared to control group.
α; Student t test
β; Chi Square test

Table 3. Endometriosis grades after surgery.

		Patient n (%)
Diagnosis	-	30 (60)
	+	20 (40)
Endometriosis grades	1	29 (58)
	2	5 (10)
	3	6 (12)
	4	10 (20)
Endometrioma	+	38 (76)
	-	12 (24)
Surgery	Laparotomy	36 (72)
	Laparoscopy	14 (28)

+: Yes; -: No; n: number.

Gal-3 has been reported to be highly expressed in ectopic, ectopic endometrium of patients with endometriosis compared to the control group (18). Especially, Gal-3 has been implicated in the development of neoplasms, including gynecological cancers (29).

The literature review revealed that Gal-1 and Gal-3 are overexpressed in the eutopic endometrium of women with endometriosis (5, 14), and Gal-3 in endometrial cells (30, 31). Based on these findings, we thought that Gal-3 expression might be abnormal in endometriosis patients.

In this pilot study, we revealed the potential for Gal-3 to be used as a biomarker in clinical practice in endometriosis. This study indicated that Gal-3 levels in the serum of women with endometriosis are remarkably increased compared with the control group. In addition, we showed that Gal-3 levels were increased in the 3 and 4 stages of endometriosis compared to the control group ($p < 0.05$). In previous studies, it was stated that Gal-3 levels increase in endometriosis. Noel et al. showed that Gal-3 expressions were increased in the proliferative or secretory phases of endometriosis compared to the eutopic

endometrium. Their study suggests that Gal-3 can play role in the development of endometriosis (18). In another study, Caserta et al. found that the expression of Gal-3 in peritoneal fluid of women with endometriosis increased significantly compared to controls (5). Mattos et al. investigated the effects of Gal-3 on the development of endometriotic lesions. They demonstrated that Gal-3 importantly contributes to endometriotic lesions development. (21). The same group showed that Gal-3 expression increased in experimental peritoneal endometriotic lesions (32). Our results support these data, Gal-3 expression levels were significantly overexpressed in stage 3-4 in endometriosis.

Even though the study includes a restricted sample and reported initial results, future studies are required to define the complete role of Gal-3 levels, and to determine biomarkers of diagnosis in endometriosis.

CONCLUSION

According to the results of this study, Gal-3 has roles in the development or progression of endometriosis, and gives us information about the severity of the disease in the advanced stages of endometriosis. However, it is clear that detailed further studies and more examples are needed to confirm these results.

Ethics Committee Approval: The study approval was obtained from the Ethics Committee of Cumhuriyet University Faculty of Medicine (No:2019-10/06).

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Conflicts of Interest: The authors declare no conflict of interest.

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