Observational Study

Medicine

OPEN

Neutrophil gelatinase-associated lipocalin serum level

A potential noninvasive biomarker of endometriosis?

Gurhan Guney, MD[a,*], Mine Islimye Taskin, MD[a], Antonio Simone Laganà, MD[b], Ezgi Tolu, MD[a], Figen Aslan, MD[c], Adnan Adil Hismiogullari, MD[d], Cihan Kaya, MD[e]

Abstract

Neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin-2) is an acute-phase protein expressed in many tissues and plays a role in cell proliferation, regulation, and epithelial-mesenchymal transformation. Therefore, this study aimed to investigate serum NGAL levels and endometrioma tissue expression in women with endometriosis. This cross-sectional study was conducted at a university hospital. The endometrioma group included 36 women who underwent ovarian cystectomy for endometrioma, which was compared with a control group (n = 36) of women who underwent ovarian cystectomy due to benign persistent cysts (follicle cyst, theca lutein cyst, and serous cystadenoma). NGAL levels were analyzed using both serum enzyme-linked immunosorbent assay analysis and immunohistochemical tissue staining. Serum C-reactive protein and CA-125 levels were also evaluated. NGAL serum levels were significantly higher in the endometrioma group than in the control group ($P < .05$). C-reactive protein and CA-125 levels were also significantly higher in the endometrioma group ($P < .05$) and were correlated with NGAL levels. Immunohistochemical staining for NGAL was also higher in the endometrioma group ($P < .001$). NGAL may be considered a potential noninvasive biomarker of endometriosis.

Abbreviations: BMI = body mass index, CRP = C-reactive protein, ELISA = enzyme-linked immunosorbent assay, NGAL = neutrophil gelatinase-associated lipocalin.

Keywords: biomarker, endometriosis, inflammation, neutrophil gelatinase-associated lipocalin, noninvasive diagnosis

1. Introduction

Endometriosis is characterized by the presence of endometrial-like tissue outside the uterine cavity, and is associated with pelvic adhesions and local inflammatory reactions.[1] Although the proposed theories cannot explain all endometriosis cases, accumulating evidence suggests a clear role of altered immune homeostasis in the peritoneal microenvironment and a consequent pro-inflammatory reaction.[2,3] Regardless of its benign character endometriosis has some characteristics of malignant neoplasms, such as local invasion, resistance to apoptosis and angiogenesis.[4]

As a complex gynecological disease with unclear etiology, endometriosis can negatively affect the quality of life.[5] To date, histopathological confirmation remains the gold standard for diagnosis, and noninvasive biomarkers have been found to be unreliable for implementation in clinical practice.[6]

In response to tissue damage and inflammation, neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is rapidly released by neutrophils. NGAL is also an acute-phase protein expressed in various tissues.[7] It plays an essential role in regulating cell proliferation and its expression has been demonstrated in many types of human cancers.[8] NGAL has also been shown to link with endometriosis pathophysiology via the epithelial-mesenchymal transition process, which is the cause of endometriosis development.[9] Moreover, NGAL, which forms a complex with matrix metalloproteinases has been demonstrated to protect matrix metalloproteinases from autodegradation in vitro. Degradation and remodeling of extracellular matrix with matrix metalloproteinases are

This study was supported by the Balikesir University Scientific Investigations Foundation (project number:2015/13).

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Reproductive Endocrinology and Infertility, Balikesir University Medical Faculty, Balıkesir, Turkey, [b] Unit of Gynecologic Oncology, ARNAS "Civico-Di Cristina-Benfratelli," Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy, [c] Department of Pathology, Balikesir University Medical Faculty, Balikesir, Turkey, [d] Department of Biochemistry, Balikesir University Medical Faculty, Balikesir, Turkey, [e] Department of Reproductive Endocrinology and Infertility, Acibadem Hospital Group, Istanbul, Turkey.

** Correspondance: Gurhan Guney, Department of Reproductive Endocrinology and Infertility, Balikesir University School of Medicine, Cagis Campus, Balıkesir 10145, Turkey (e-mail: gurhanguney@yahoo.com).*

How to cite this article: Guney G, Taskin MI, Laganà AS, Tolu E, Aslan F, Hismiogullari AA, Kaya C. Neutrophil gelatinase-associated lipocalin serum level: A potential noninvasive biomarker of endometriosis? Medicine 2023;102:41(e35539).

Received: 27 October 2022 / Received in final form: 20 August 2023 / Accepted: 15 September 2023

http://dx.doi.org/10.1097/MD.0000000000035539

essential processes during cellular migration and tissue invasion in the pathophysiology of endometriosis.[10]

Based on the above relationships, we aimed to evaluate NGAL as a potential biomarker of endometriosis and also its associations with Ca-125 and C-reactive protein (CRP). We belive that our study will make a positive contribution to the endometriosis researches and literature.

2. Materials and Methods

2.1. Study design

This cross-sectional study was approved by our institutional review board (approval number:2015/13) prior to enrollment. The study was conducted between November 2015 and August 2017, in agreement with the Declaration of Helsinki, the Committee on Publication Ethics guidelines (http://publicationethics.org/), and the Strengthening the Reporting of Observational Studies in Epidemiology statement available through the (enhancing the quality and transparency of health research) network (www.equator-network.org).

Each patient enrolled in this study signed an informed consent form for all procedures and allowed data collection and analysis for research purposes. The study was non-advertised, and no remuneration was offered to encourage the patients to provide consent. In our sample size analysis using GPower software (Universität Kiel, Kiel, Germany), we found that at least 32 patients in each group ($\alpha = 0.05$, effect size = 0.80) were required to achieve a power of 95 % ($1\text{-}\beta = 0.95$). We randomly selected the study participants to avoid bias.

2.2. Study participants

Seventy-Two reproductive aged (20–45 years) who underwent surgery for endometrioma or other ovarian cysts were included in the study. Demographic characteristics such as age, body mass index (BMI), gravidity, parity, smoking habit, education level, and presenting symptoms were recorded. The patients were then divided into 2 groups. The endometrioma group (n = 36) included women who underwent enucleation of ovarian endometrioma and were classified according to the revised American Fertility Society as stage III to IV endometriosis. The control group (n = 36) included women who had undergone ovarian cystectomy for benign persistent cysts (follicle cysts, theca lutein cysts, and serous cystadenomas). We evaluated ultrasonographically that the patients had cysts other than endometrioma, and we found that they did not have endometrioma intraoperatively during the operation. In addition, we examined and confirmed microscopically on the pathology samples that there was no concomitant endometriosis in the cystectomy materials during the surgery.

The exclusion criteria were underweighted (BMI < 19 kg/m^2), obesity (BMI > 30 kg/m^2), hormonal therapy in the last 3 months (wash-out period), the presence of acute or chronic inflammation, autoimmune diseases, and malignancy. NGAL levels were analyzed immunohistochemically in biopsies taken from cyst capsules during surgery. In addition, NGAL levels were determined by enzyme-linked immunosorbent assay (ELISA) in serum samples obtained from venous blood samples before the start of surgery, which is always performed in the proliferative phase of the menstrual cycle. Serum levels of CRP and CA-125 were evaluated using ELISA.

2.3. Biochemical analyses

Serum samples were separated by centrifugation at 825g for 10 minutes. for biochemical analysis. Serum NGAL levels were determined by ELISA using commercially available kits (Bioassay Technology Laboratory, China).

2.4. Immunohistochemical method

Paraffin blocks of the tissue samples were evaluated. Tissue sections of 3 to 4 micron thickness were obtained from the paraffin blocks. The slides were then placed into a cabin (Ventana Benchmark XT) to dissolve the paraffin. Then, CC1 (Cell Conditioner 1) solution was applied to the slides to reveal the antigen. Hydrogen peroxide was applied for 7 minutes. to mask for endogenous peroxidases. Subsequently, the primary antibody NGAL (Boster Bio-polyclonal anti-lipocalin-2 picoband antibody, 1/1000) was added and incubated for 30 minutes. The secondary antibody of the Ultra View Universal DAB Detection kit was applied for 10 minutes. Counterstaining was performed using hematoxylin for 8 minutes. and then finalized as background staining with bluing reagent solution. The slides that were taken from the cabin were dehydrated with alcohol, made transparent with xylene, and covered with a coverslip.

2.5. Immunohistochemical evaluation

To prevent inter-individual bias, all tissues were evaluated by the same pathologist who was blinded to the origin of the samples. For immunohistochemical staining, semiquantitative evaluation was performed based on the intensity of staining, as shown in Figure 1. The intensity of staining was scored between 0 to 3 points according to the expression in the cytoplasm of epithelial cells (no staining,0; weak staining,1; moderate staining,2; strong staining,3 points). To evaluate the staining score, 0 to 1: accepted as negative and 2 to 3: was accepted as positive, and mean histological scores between the groups were compared using a bar graph in **Figure 2**.

2.6. Statistical analysis

The data analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL). The following descriptive statistics were used to define the study data: mean, median, standard deviation, and frequency. The Mann–Whitney *U* test and Spearman correlation coefficient were used to compare study variables. Statistical significance was set at $P < .05$.

3. Results

The demographic characteristics of the study groups are presented in Table 1. There were no significant differences between the study groups in terms of age ($P = .11$), smoking habits ($P = .20$), and BMI ($P = .16$). Gravidity and parity ($P = .003$) were significantly higher in the control group than in the endometriosis group ($P = .007$ and 0.003, respectively). Symptoms, such as pain and infertility, were significantly higher in the endometriosis group than in the control group ($P = .004$).

Differences in serum NGAL, CRP, and CA-125 levels and immunohistochemical scores between the groups are shown in Table 2. Serum NGAL levels were significantly higher in the endometriosis group than in the control group ($P < .001$). In addition, CRP and CA-125 levels and immunohistochemical scores were significantly higher in patients with endometriosis ($P < .05$, for all comparisons). The results are shown in Figure 3. Correlation analysis between NGAL and other parameters showed a positive correlation (Table 3) between NGAL and CA-125 ($P < .001$; $R = 0.72$), immunohistochemical score ($P < .001$; $R = 0.53$), and CRP ($P < .001$, $R = 0.48$).

4. Discussion

Our analysis showed that serum NGAL levels were significantly higher in patients with endometriosis. Moreover, a significant correlation was observed between NGAL levels and CRP, CA-125, and immunohistochemical scores.

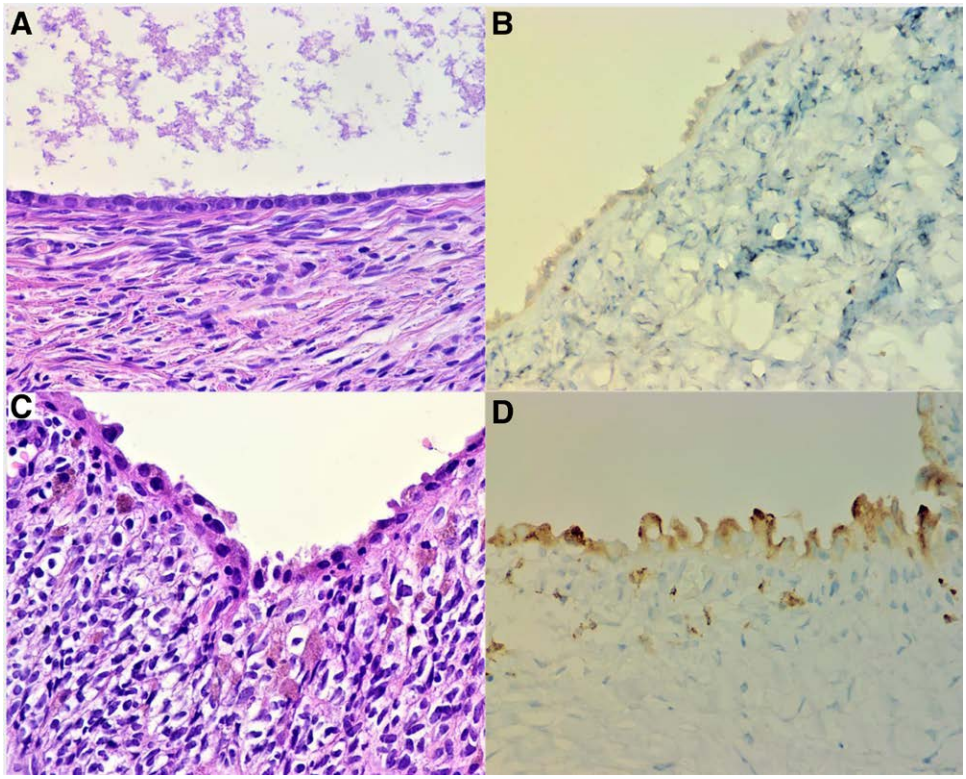

Figure 1. (A) Serous ovarian cyst (H&Ex400), (B) NGAL expression in serous ovarian cyst (NGALx400), (C) endometriotic cyst (H&Ex400), and (D) NGAL expression in endometriotic cyst (NGALx400). NGAL = neutrophil gelatinase-associated lipocalin.

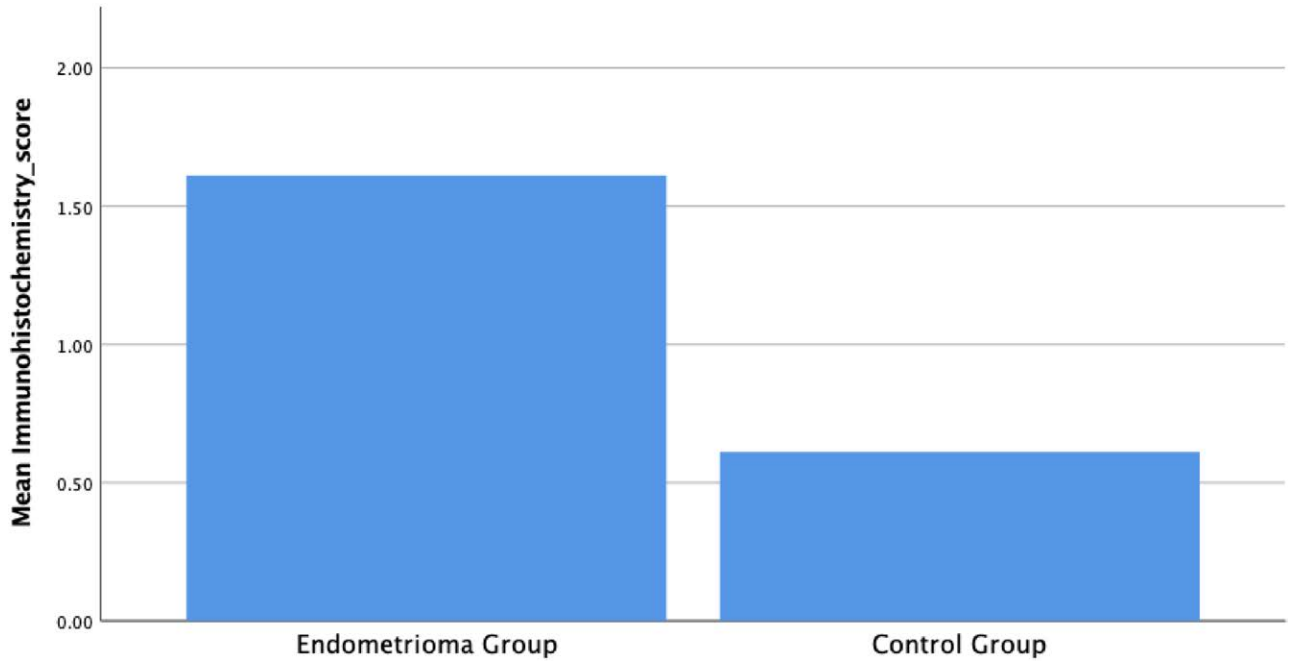

Figure 2. Comparision of mean immunohistochemistry scores between endometrioma and control group (1.61 ± 0.54 endometrioma group vs 0.61 ± 0.80 control group, $P < .001$).

In a comprehensive retrospective cohort study, 15,488 endometriosis patients with endometriosis were evaluated considering the coexistence of endometrial cancer. The incidence of endometrial cancer in the endometriosis group was 2.9 times higher than that in the control group. In addition, estrogen hormones and chronic inflammation may account, at least in part, for the relationship between endometriosis and endometrial cancer.[11]

The loss of cellular contact and subsequent implantation and proliferation are related to the pathogenesis of endometriosis, and

NGAL may be a reliable biomarker for these events in the peritoneal microenvironment.[12–14] Liao et al[9] reported that NGAL could enable the transformation of epithelial cells into mesenchymal cells, one of the initial steps of endometriosis. Moreover, they also suggested that increased NGAL and decreased cytokeratin levels might cause further tissue adhesion and account for disease progression. Joseph et al reported that NGAL may be associated with the transformation of epithelial cells into mesenchymal cells in human peritonitis models.[15] In contrast, Durmus et al[16] evaluated serum NGAL levels in patients with endometriosis and reported no significant difference compared to control patients. They also reported higher NGAL levels in stage I-II patients than stage III-IV patients (3.6 vs 1.2 ng/dL). However, they did not evaluate tissue NGAL expression as we did. Indeed, our study found significantly higher NGAL levels in serum and tissue samples in the endometriosis group than in the controls with persistent benign ovarian cysts. Based on our results, we speculate that NGAL may play a role in endometriosis pathogenesis.

Table 1

Demographic characteristics of the patients.

		Endometrioma group (n = 36)	Control group (n = 36)	*P* value
Age (yr)		31,3 ± 3,78	29,5 ± 4,29	.11
Gravidity		0.25 ± 0,690	0.75 ± 1,07	.007
Parity		0.22 ± 0,59	0.58 ± 0,77	.003
Smoking	Yes n (%)	8 (22%)	13 (36%)	.20
	No n (%)	28 (78%)	23 (64%)	
BMI (kg/m^2)		25,9 ± 3,75	24,3 ± 3,55	.16
Symptoms	Pain n (%)	22 (62%)	16 (44%)	.004
	Infertility n (%)	10 (28%)	2 (6%)	
	Other n (%)	1 (2%)	8 (22%)	

Data are expressed as means ± standard deviations for continuous variables, and as n (%) for dichotomous variables.
BMI = body mass index.

Table 2

Comparison of neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP), CA-125 and immunohistochemistry score between endometriosis group and controls.

	Endometrioma group (n = 36)	Control group (n = 36)	*P* value
NGAL (ng/dL)	194.09 ± 51.57	57.69 ± 29.52	<.001
CRP (mg/L)	7.95 ± 3.91	4.66 ± 2.37	<.001
CA-125 (UI/mL)	45.60 ± 12.56	14.88 ± 6.58	<.001
Immunohistochemistry score	1.61 ± 0.54	0.61 ± 0.80	<.001

Data are expressed as mean ± standard deviations.
CRP = C-reactive protein, NGAL = neutrophil gelatinase-associated lipocalin.

Kobayashi et al,[17] they evaluated dienogest-resistant and dienogest-sensitive endometrioma cases. They reported that NGAL might be an extracted protein, especially in cases of drug-resistant endometriosis. They also hypothesized that the pro-inflammatory microenvironment within endometriotic cysts might be a cause of ovarian cancer development, and NGAL might play a role in this event because it interacts with other growth factors responsible for ovarian cancer formation.

There is a need to understand the molecular changes in chronic diseases, such as endometriosis, which is challenging to treat due to its chronic course and local aggressive pattern.[18,19] In a study evaluating NGAL expression via immunohistochemistry, a strong increase in expression was observed in patients with endometrial hyperplasia.[20] In another study, Miyamoto et al[21] showed that the immunohistochemical expression of NGAL protein was increased in higher grade and advanced stage endometrioid-type endometrial cancer. They also found that NGAL is involved in promoting endometrial cell migration and survival against cisplatin and UV irradiation.[21]

In our study, we evaluated the immunostaining score for NGAL expression for the first time in the literature and found that NGAL expression was significantly higher in endometriosis cases than in the control group. As an example of the immunohistochemical scoring study similar to our study, Löffelman AC et al[22] investigated the expression of Claudin 10 in a study

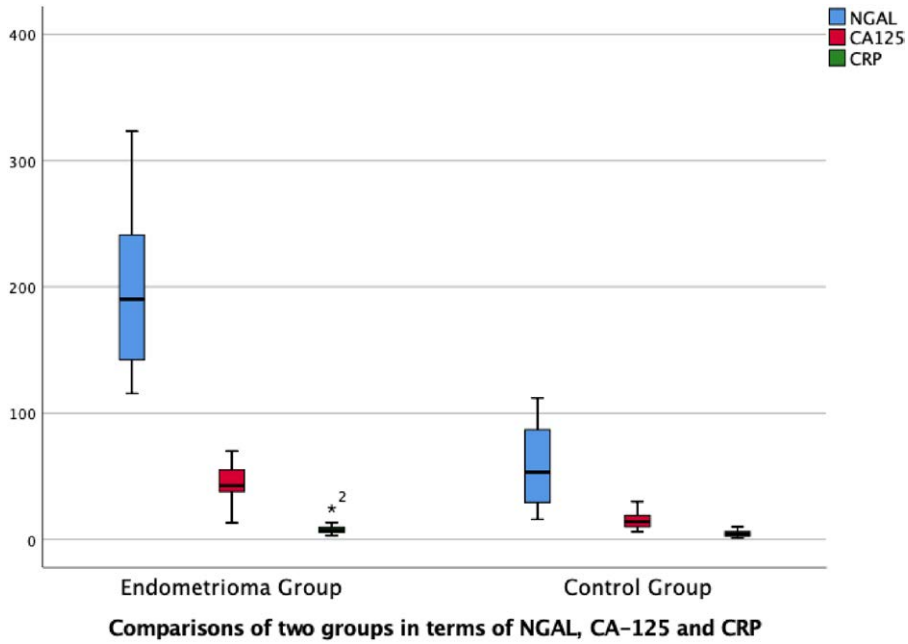

Figure 3. Box plot analysis of NGAL, Ca-125 and CRP between endometrioma and control group. (*P* < .001 for NGAL,Ca-125 and CRP). CRP = C-reactive protein, NGAL = neutrophil gelatinase-associated lipocalin.

Table 3

Correlation analysis of the study variables.

	CRP (mg/L)		CA-125 (U/mL)		Immunohistochemistry score	
	r	*P* value	r	*P* value	r	*P* value
NGAL (ng/mL)	0.485	<.001	0.721	<.001	0.534	<.001

CRP = C-reactive protein, NGAL = neutrophil gelatinase-associated lipocalin, r = correlation coefficient.

on human endometrium, endometriosis and adenomyosis tissue samples. Claudins are the major component of tight junctions and play a role in the escape of cancer cells out of the tissue. They found that the immunohistochemical score values for Claudin 10 were significantly higher in deeply infiltrating endometriosis, peritoneal endometriosis, and especially ovarian endometriosis.

Early diagnosis and treatment of endometriosis both prevent the development of malignancy and endometriosis-related complications such as infertility and pain. Therefore, it is important to establish the diagnosis with new, rapid and noninvasive biomarkers. In a comprehensive review by Bommi JR et al,[23] electrochemical and optical biosensors, which were specifically developed to detect the levels of frequently used cancer biomarkers markers, are very exciting and bring the advantage of being able to intervene in diseases such as endometriosis in early period.

Although research on markers for the diagnosis of endometriosis continues, CA-125 is one of the most investigated tumor markers for the diagnosis of endometriosis, although its evaluation is not recommended in most of the available international guidelines because of its low sensitivity. Although its increase could be associated with gynecological and non-gynecological reasons, CA-125 levels commonly increase in endometriosis cases.[24–26].

Our study found that CA-125 levels were significantly higher in endometriosis patients than in controls, in line with the available literature. Interestingly, we also detected a positive correlation between CA-125 and NGAL levels, and histological scores. Although NGAL levels are elevated in some types of cancers, we observed that the number of studies investigating the relationship between NGAL levels and any tumor markers was not so much.[27] In the study conducted by Hogendorf P et al,[28] which is one of the studies investigating this relationship, it was stated that ca 19 to 9 and CA-125 levels, when the levels are examined to differentiate chronic pancreatitis and pancreatic adenocarcinoma, may not always predict pancreatic adenocarcinoma. They also stated that if the high levels of Ca-125 and Ca 19 to 9 are accompanied by high levels of NGAL, this elavation was secondary to pancreatic adenocarcinoma with a higher probability rather than chronic pancreatitis. In another study by Coticchia et al,[29] patients with ovarian cancer with normal Ca-125 levels were investigated and it was observed that NGAL levels in the urine of these patients were found to be high together with some matrix metalloproteinases, and it was mentioned that NGAL could be used as a tumor marker in some types of ovarian cancer. Both studies mentioned above are important in that they showed the relationship of NGAL with CA-125 and CA 19 to 9, which are tumor markers that we use frequently in gynecology practice.

One of the main limitations of our study could be the small number of the study population; second, we did include only women with advanced stage of the disease; third, the endometriosis tissue sample were obtained only from endometrioma, so we do not have data about the expression in deep infiltrating endometriosis; finally, all the blood samples were collected before surgery which was scheduled always in the proliferative phase, so we could not draw firm conclusion about the potential fluctuation of NGAL levels within the menstrual cycle. Nevertheless, to the best of our knowledge, this is the first study to investigate NGAL levels in endometriosis using serum analysis and immunohistochemical expression.

5. Conclusion

Our results demonstrate that NGAL may play a role in endometriosis pathogenesis, although further studies in larger populations are needed to investigate its potential as a biomarker.

Acknowledgements

We would like to thank all patients who agreed to participate in this study.

Author contributions

Conceptualization: Gurhan Guney, Mine Islimye Taskin.
Data curation: Gurhan Guney, Ezgi Tolu.
Formal analysis: Gurhan Guney, Ezgi Tolu, Figen Aslan, Adnan Adil Hismiogullari.
Funding acquisition: Mine Islimye Taskin.
Investigation: Gurhan Guney, Figen Aslan.
Methodology: Gurhan Guney.
Project administration: Mine Islimye Taskin.
Resources: Gurhan Guney, Figen Aslan, Adnan Adil Hismiogullari.
Software: Gurhan Guney.
Supervision: Gurhan Guney, Antonio Simone Lágana, Cihan Kaya.
Validation: Gurhan Guney, Antonio Simone Lágana.
Visualization: Gurhan Guney, Antonio Simone Lágana.
Writing – original draft: Gurhan Guney.
Writing – review & editing: Gurhan Guney, Mine Islimye Taskin, Cihan Kaya.

References

[1] Missmer SA, Hankinson SE, Spiegelman D, et al. Reproductive history and endometriosis among premenopausal women. Obstet Gynecol. 2004;104(5 Pt 1):965–74.
[2] Laganà AS, Garzon S, Götte M, et al. The pathogenesis of endometriosis: molecular and cell biology insights. Int J Mol Sci. 2019;20:5615.
[3] Laganà AS, Vitale SG, Salmeri FM, et al. Unus pro omnibus, omnes pro uno: a novel, evidence-based, unifying theory for the pathogenesis of endometriosis. Med Hypotheses. 2017;103:10–20.
[4] Monnin N, Fattet AJ, Koscinski I. Endometriosis: update of pathophysiology, (epi) genetic and environmental involvement. Biomedicines. 2023;11:978.
[5] Soliman AM, Coyne KS, Zaiser E, et al. The burden of endometriosis symptoms on health-related quality of life in women in the United States: a cross-sectional study. J Psychosom Obstet Gynaecol. 2017;38:238–48.
[6] Brasil DL, Montagna E, Trevisan CM, et al. Psychological stress levels in women with endometriosis: systematic review and meta-analysis of observational studies. Minerva Med. 2020;111:90–102.
[7] Vitale SG, La Rosa VL, Rapisarda AMC, et al. Impact of endometriosis on quality of life and psychological well-being. J Psychosom Obstet Gynaecol. 2017;38:317–9.
[8] Vercellini P, Viganò P, Somigliana E, et al. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10:261–75.

[9] Liao CJ, Li PT, Lee YC, et al. Lipocalin 2 induces the epithelial-mesenchymal transition in stressed endometrial epithelial cells: possible correlation with endometriosis development in a mouse model. Reproduction. 2014;147:179–87.
[10] Becker CM, Louis G, Exarhopoulos A, et al. Matrix metalloproteinases are elevated in the urine of patients with endometriosis. Fertil Steril. 2010;94:2343–6.
[11] Yu HC, Lin CY, Chang WC, et al. Increased association between endometriosis and endometrial cancer: a nationwide population-based retrospective cohort study. Int J Gynecol Cancer. 2015;25:447–52.
[12] Mendonsa AM, Na TY, Gumbiner BM. E-cadherin in contact inhibition and cancer. Oncogene. 2018;37:4769–80.
[13] Painter JN, O'Mara TA, Morris AP, et al. Genetic overlap between endometriosis and endometrial cancer: evidence from cross-disease genetic correlation and GWAS meta-analyses. Cancer Med. 2018;7:1978–87.
[14] Xu H, Sun X, Sun WJ. Expression and clinical correlation of NGAL and VEGF in endometrial carcinoma. Eur Rev Med Pharmacol Sci. 2018;22:632–6.
[15] Leung JC, Lam MF, Tang SC, et al. Roles of neutrophil gelatinase-associated lipocalin in continuous ambulatory peritoneal dialysis-related peritonitis. J Clin Immunol. 2009;29:365–78.
[16] Bostanci Durmus A, Dincer Cengiz S, Yılmaz H, et al. The levels of matrix metalloproteinase-9 and neutrophil gelatinase-associated lipocalin in different stages of endometriosis. J Obstet Gynaecol. 2019;39:991–5.
[17] Kobayashi Y, Fukutomi T, Mita S, et al. Identification of biomarkers for drug-resistant endometriosis using clinical proteomics. Hum Cell. 2021;34:394–9.
[18] Nothnick WB, Marsh C, Alali Z. Future directions in endometriosis research and therapeutics. Curr Womens Health Rev. 2018;14:189–94.
[19] Zaha DC. Significance of immunohistochemistry in breast cancer. World J Clin Oncol. 2014;5:382–92.
[20] Liao CJ, Huang YH, Au HK, et al. The cancer marker neutrophil gelatinase-associated lipocalin is highly expressed in human endometrial hyperplasia. Mol Biol Rep. 2012;39:1029–36.
[21] Miyamoto T, Asaka R, Suzuki A, et al. Immunohistochemical detection of a specific receptor for lipocalin2 (solute carrier family 22 member 17, SLC22A17) and its prognostic significance in endometrial carcinoma. Exp Mol Pathol. 2011;91:563–8.
[22] Loeffelmann AC, Hoerscher A, Riaz MA, et al. Claudin-10 expression is increased in endometriosis and adenomyosis and mislocalized in ectopic endometriosis. Diagnostics (Basel). 2022;12:2848.
[23] Rao Bommi J, Kummari S, Lakavath K, et al. Recent trends in biosensing and diagnostic methods for novel cancer biomarkers. Biosensors (Basel). 2023;13:398.
[24] O DF, Flores I, Waelkens E, et al. Noninvasive diagnosis of endometriosis: review of current peripheral blood and endometrial biomarkers. Best Pract Res Clin Obstet Gynaecol. 2018;50:72–83.
[25] Karimi-Zarchi M, Dehshiri-Zadeh N, Sekhavat L, et al. Correlation of CA-125 serum level and clinico-pathological characteristic of patients with endometriosis. Int J Reprod Biomed. 2016;14:713–8.
[26] Kaya C, Cengiz H, Ekin M, et al. Transverse vaginal septum: a benign reason for elevated serum CA 19-9 and CA 125 levels. Arch Gynecol Obstet. 2012;286:821–3.
[27] Roli L, Pecoraro V, Trenti T. Can NGAL be employed as prognostic and diagnostic biomarker in human cancers? a systematic review of current evidence. Int J Biol Markers. 2017;32:e53–61.
[28] Hogendorf P, Durczyński A, Skulimowski A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) concentration in urine is superior to CA19-9 and Ca 125 in differentiation of pancreatic mass: preliminary report. Cancer Biomark. 2016;16:537–43.
[29] Coticchia CM, Curatolo AS, Zurakowski D, et al. Urinary MMP-2 and MMP-9 predict the presence of ovarian cancer in women with normal CA125 levels. Gynecol Oncol. 2011;123:295–300.