ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20230613

Role of cystatin C levels as an inflammatory marker in predicting endometriosis

Harun Kılıçkıran[1*], İnci Halilzade[1], Mohammad İbrahim Halilzade[1], Canan Topçuoğlu[2], Mehmet Çınar[1]

SUMMARY

OBJECTIVE: Endometriosis is a common chronic inflammatory disease associated with infertility and pelvic pain. Diagnosis is based on the appearance of endometriotic lesions at the time of surgery. Our study aimed to determine whether cystatin C can be used as a predictor of endometriosis and to investigate its potential role in doing so.

METHODS: The study included 45 patients with endometriosis between the ages of 18 and 40 years whose pathology results were compatible with endometriosis and were operated on, and a control group of 45 healthy women. These two groups were compared in terms of serum cystatin C levels, demographic-clinical characteristics, operation results, and other laboratory values.

RESULTS: The cystatin C and hs-CRP levels of the endometriosis patients were found to be significantly higher than the control subjects ($p<0.005$). Whether the endometriosis disease could be detected for serum cystatin C levels was determined by the receiver operating characteristic analysis and the most appropriate positive cutoff value for cystatin C was found to be 5.14 ng/mL (86.7% sensitivity and 77.8% specificity). In the linear regression analysis, it was observed that the probability of endometriosis increased 2.5 times when cystatin C levels increased above the threshold value of 5.14 ng/mL (OR: 2.5; 95%CI 2.24–2.76).

CONCLUSION: Our study shows that the serum cystatin C levels can be used as a guide for diagnosis in patients with advanced endometriosis. However, more research is needed to prove its reliability and accuracy in order to put it into practice.

KEYWORDS: Endometriosis. Endometrioma. Cystatin C. Diagnosis. Anti-inflammatory agents.

INTRODUCTION

Endometriosis, which is a common chronic inflammatory disease associated with infertility and pelvic pain, is characterized by the presence of endometrium-like glands and tissues outside the uterus[1]. Currently, diagnosis relies on visualizing endometriotic lesions during surgery, as there is no reliable serum marker available[2]. Moreover, the origin of endometriosis is still largely unknown[1]. Therefore, medical history and biochemical markers were investigated together with ultrasonographic methods as an alternative to invasive methods in diagnosis[3]. Although the role of various gene expressions has been demonstrated in recent studies, serum biomarkers (e.g., TNF-α, IL-6, IL-1β, CA-125, and CA 19-9) remain uncertain as suitable candidates for non-invasive methods, even though they may be suitable candidates for non-invasive methods[4]. Furthermore, one study revealed an increased prevalence of endometriosis during the severe acute respiratory syndrome coronavirus 2 pandemic, raising more questions regarding the etiogenesis of endometriosis[5].

Cystatin C is a cysteine protease inhibitor produced by all nucleated cells. It is thought that cystatin C reduces endogenous cysteine protease and neutrophil migration activity in the inflammatory process[6]. Cystatin C is an important marker, especially in demonstrating kidney function and glomerular filtration rate[7]. However, recent studies have revealed the significance of cystatin C in various fields. In addition to studies suggesting that it is an early predictor of cardiovascular diseases[8], some studies show that it can be a good biomarker for cerebrovascular diseases and peripheral vascular diseases[9]. Furthermore, studies have reported that cystatin C shows renal damage in patients with preeclampsia[10]. However, as cystatin C is considered to be a predictor of inflammation, it has been shown to be associated with malignancies. Cystatin C has been shown to be associated with various malignancies, in particular, urogenital malignancies[11]. However, no study in the literature investigates whether it is a suitable biomarker for endometriosis. In this respect, this study is significant in that it is the first of its kind and will make a valuable contribution to the literature.

Hence, the aim of our study was to search for a non-invasive method that could assist in the diagnosis of endometriosis, the etiogenesis of which is still unclear, and to investigate the role of cystatin C in predicting endometriosis.

[1]University of Health Sciences, Ankara City Hospital, Department of Obstetrics and Gynecology – Ankara, Turkey.
[2]University of Health Sciences, Ankara City Hospital, Department of Biochemistry – Ankara, Turkey.
*Corresponding author: harunkilickiran@hotmail.com
Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on July 24, 2023. Accepted on August 26, 2023.

METHODS

Our study included 90 women, 45 of whom were volunteer patients between the ages of 18 and 40 years who applied to the city hospital endometriosis polyclinic between January 2022 and June 2022, and the other 45 patients were in the control group who applied to the gynecology polyclinic. Our study was conducted in accordance with the Declaration of Helsinki and in compliance with the country's ethical standards. Ethics committee approval was obtained from the same hospital (21/1046). An informed consent form was signed by all patients. Endometriosis patients were selected using the revised American Fertility Society classification as patients who had undergone surgery for pelvic pain or infertility and whose pathology results were compatible with endometriosis. The control group was selected from healthy women volunteers between the ages of 18 and 40 years without infertility and no additional diseases. Patients who were pregnant and had gynecological comorbidities, active infections, kidney disease, cardiovascular disease, cerebrovascular disease, malignancy, and chronic autoimmune diseases were not included in the study.

The demographic characteristics, obstetric histories, body mass index (BMI) values, ultrasonographic findings, physical examination findings, pathology results, and serum biochemical and hormonal parameters (hemoglobin (Hb), white blood cell (Wbc), neutrophil, lymphocyte, sodium, potassium, AST, ALT, urea, creatinine, hs-CRP, procalcitonin, CA-125, anti-Müllerian hormone (AMH), and cystatin C levels) of each patient were recorded. When calculating BMI, the patients' height and weight were measured, and it was calculated using the formula: BMI=weight (kg)/height $(m)^2$. All these parameters were compared between the endometriosis and control groups.

Cystatin C levels were measured using a commercial ELISA kit (Elabscience, Elabscience Biotechnology Co. Ltd. Wuhan, P.R.C., Catalog No: E-EL-H3643, LOT: ER04688F5606). The measurement range is 0.31–20 ng/mL. Its sensitivity is 0.19 ng/mL, and the intra-assay and inter-assay %CV values are <10%. In the endometriosis group, serum cystatin C levels were taken preoperatively. Blood samples were collected in yellow-capped, vacuum-sealed, plastic gel tubes from both the endometriosis and control groups between 8:00 a.m. and 12:00 p.m. after 12 h of fasting.

Statistical analyses were performed using SPSS version 22. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed using the mean and standard deviations for normally distributed variables. The means of parametric data determined by Levene's test, which showed normal distribution, were compared using Student's t-test. The Mann-Whitney U test was used to compare the parametric and ordinal data, which were determined not to be normally distributed. The presence of correlation between parametric data was tested using the Pearson test, and the presence of correlation between nonparametric and non-normal distributed data was tested using the Spearman test. Categorical data were compared using chi-square or Fisher's exact test (where values observed in cells did not meet the chi-square test assumptions) as appropriate. Cases with a $p<0.05$ were considered statistically significant. The role of cystatin C in predicting endometriosis was investigated using the ROC curve analysis method.

RESULTS

The comparison of demographic characteristics and biochemical and hormonal parameters between the endometriosis and control groups is shown in Table 1. While there was no significant difference between the groups in terms of mean age and BMI, gravida and parity variables were found to be significantly lower in the endometriosis group. When serum cystatin C levels were compared, a statistically significant difference was found between the endometriosis and control cases ($p<0.001$). Moreover, the hs-CRP ($p=0.002$) and CA-125 ($p<0.001$) values of endometriosis patients were found to be significantly higher than those of the control subjects (Table 1).

According to the ROC curve analysis (Figure 1), the cystatin C level was a discriminating parameter in patients with endometriosis. The area under the curve for cystatin C was 0.92 (0.86–0.98) at 95% confidence interval (Figure 1). The threshold value for cystatin C was 5.14 ng/mL with a sensitivity of 86.7% and a specificity of 77.8%.

In the linear regression analysis, it was observed that the probability of endometriosis increased 2.5 times when cystatin C levels exceeded the 5.14 ng/mL threshold (OR: 2.5; 95%CI 2.24–2.76) (Table 2).

DISCUSSION

This study evaluated the possible association between serum cystatin C levels and endometriosis, and to the best of our knowledge, this is the first study to evaluate the relationship between endometriosis and the proinflammatory marker cystatin C. In our study, serum cystatin C levels were found to be statistically significantly higher in the endometriosis group when compared with the control group ($p<0.001$). Furthermore, the hs-CRP ($p=0.002$) and CA-125 ($p<0.001$) values of endometriosis patients were found to be significantly higher than those of the control subjects. According to the ROC curve analysis, cystatin

C levels were a distinctive parameter in patients with endometriosis. The area under the curve for cystatin C was 0.92 (0.86–0.98) at 95%CI. The threshold value for cystatin C was found to be 5.14 ng/mL, with a sensitivity of 86.7% and a specificity of 77.8%. In the linear regression analysis, it was observed that the probability of endometriosis increased by 2.5 times when cystatin C levels increased above the 5.14 ng/mL threshold (OR: 2.5; 95%CI 2.24–2.76).

As cystatin C is a protease inhibitor that plays a role in inflammatory processes, it has been investigated in many diseases associated with inflammation[7-11], but research in the field of obstetrics and gynecology is limited. In one meta-analysis, it was demonstrated to be a promising biomarker in the detection of preeclampsia[12]. Additionally, another study reported that serum cystatin C levels in late pregnancy were associated with negative birth outcomes[13]. On the contrary, Zhang et al., suggested that serum cystatin C levels are significantly higher in patients with gestational diabetes mellitus (GDM) compared

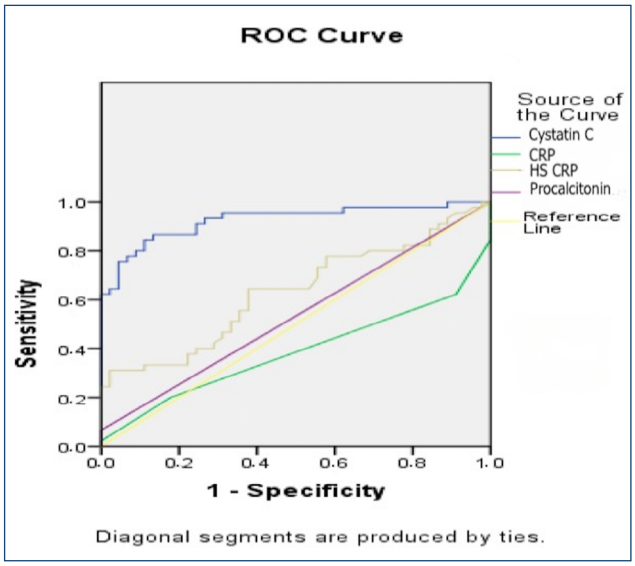

Figure 1. The receiver operating characteristic (AUC: 0.926; p=0.000; 95%CI 0.869–0.983) demonstrates the diagnostic potential of "Cystatin C" and "procalcitonin" as a variable for endometriosis.

Table 1. Comparison of demographic characteristics and biochemical and hormonal parameters in endometriosis and control groups.

	Endometriosis n=45 mean±std	Control n=45 mean±std	p-value
Age (years)	31.67±6.85	31.33±5.43	0.79
Gravida	1.02±1.45	2±0.87	<0.001
Parity	0.73±1.09	1.64±0.645	<0.001
BMI (kg/m^2)	24.66±1.97	24.36±2.10	0.484
Cystatin-C (ng/mL)	10.93±5.79	3.84±2.05	<0.001
HB (g/dL)	12.5±1.17	13.0±1.27	0.057
WBC (×10^9/L)	7.42±1.98	7.33±1.67	0.814
Neutrophil (×10^9/L)	5.64±6.49	4.39±1.37	0.212
Lymphocyte (×10^9/L)	2.02±0.59	2.19±0.55	0.173
N/L	2.99±3.71	2.13±0.99	0.136
Sodium (mEq/L)	139±1.89	138±1.76	0.078
Potassium (mEq/L)	4.97±0.65	4.22±0.26	0.376
AST (U/L)	19.8±6.98	20.48±4.08	0.582
ALT (U/L)	19.8±9.24	17.71±7.1	0.219
Urea (mg/dL)	24.7±5.55	24.3±6.48	0.781
Creatinine (mg/dL)	0.65±0.09	0.66±0.13	0.883
hs-CRP (mg/L)	3.0±3.20	1.35±1.19	0.002
CRP (g/L)	0.26±0.11	0.03±0.005	0.031
Procalcitonin (μg/L)	0.38±0.37	0.03±0.00	0.133
CA-125 (U/mL)	81.5±54.22	11.24±0.00	<0.001
AMH (ng/mL)	3.06±2.01	3.39±1.82	0.421

N/L: neutrophil-lymphocyte ratio.

Table 2. Linear regression analysis and results.

	Endometriosis		
Variables	**Level (ng/mL)**	**OR (95%CI)**	**p**
Cystatin C	5.14	2.5 (2.24–2.76)	<0.001

with the control group[14]. A study reported that increased serum cystatin C levels may be a risk factor for pregnant women with PCOS and GDM[15].

Studies on cystatin C in gynecology in the literature are mostly related to polycystic ovary syndrome (PCOS), and one study stated that cystatin C levels were significantly higher in women with PCOS compared with the control group and could be an important indicator for reducing cardiovascular risks[16]. Moreover, a study conducted in adolescents with PCOS suggested that the risk of PCOS increased 1.556 times when cystatin C increased by one unit and that there was a significant relationship between them[17]. Another study in patients with adolescent PCOS suggested that cystatin C may be a promising indicator in predicting future metabolic risks[18].

Various cytokines and markers have been shown in both the peritoneal cavity and serum in patients with endometriosis[4], but the question of their role in the development of endometriosis and whether they are the cause or the result of endometriosis has not yet been clearly elucidated. In addition, although there is a difference between superficial and deep endometriosis, studies have shown that biomarkers contribute to the diagnosis of both superficial and deep pelvic endometriosis[4]. Although serum CA-125 is the most studied marker, studies have shown that its diagnostic performance is poor[19]. A meta-analysis conducted by Sokolov et al., stated that other markers such as CA 19-9 and CA 72-4 are more valuable in differentiating endometriosis from other pathologies and may help clarify the effect of circulating micro-RNA in the pathology of endometriosis[20]. Furthermore, a study investigating the role of autoantibodies and enzymes in the diagnosis of endometriosis suggested that autoantibodies against tropomyosin 3, α-enolase, and estradiol could be included in the panel of biomarkers for the non-invasive diagnosis of endometriosis[21]. Another study evaluated the hormonal etiologies of endometriosis and showed that, in rat models, gestrinone antagonizes the effects of estrogen on rat peritoneal endometrial implants when given combined estrogen therapy with gestrinone[22].

In their research, Soto et al., stated that numerous potential biomarkers for non-invasive tests for endometriosis, including glycoproteins, inflammatory cytokines, immune molecules, angiogenesis factors, hormones, microRNAs (miRNAs), proteomics, metabolomics, genomics, and microbiomes, have been investigated. However, they explained that the most promising and progressing areas for the non-invasive diagnosis of endometriosis are miRNAs, proteomics, metabolomics, genomics, and microbiome[23]. A study investigating the genetic origin of endometriosis compared the expression of stem cell-related genes in the endometrium, superficial endometriosis, and deep infiltrating endometriosis. It has been revealed that deep and superficial endometriosis tissues have similar stem cell-related genes; however, there are differences in gene expression between them[24]. Despite all these studies, a recent study stated that more confirmatory studies are required to fully establish these markers in the diagnosis, progression, and staging of endometrial lesions[25]. Many markers have been studied and continued to be investigated for the non-invasive diagnosis of endometriosis. The strengths of this study are as follows: this is the first study in the literature showing the relationship between endometriosis and cystatin C and diagnosis of endometriosis was supported by pathological examination in all patients. The limitations of our study are the small number of participants and its non-randomized design.

CONCLUSION

Cystatin C levels seem to be a promising non-invasive indicator in predicting endometriosis associated with inflammation.

AUTHORS' CONTRIBUTIONS

HK: Conceptualization, Writing – original draft. **İH**: Investigation, Writing – review & editing. **MİH**: Data curation, Methodology. **CT**: Data curation, Methodology. **MÇ**: Supervision, Formal Analysis.

REFERENCES

1. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol. 2019;15(11):666-82. https://doi.org/10.1038/s41574-019-0245-z
2. Koninckx PR, Fernandes R, Ussia A, Schindler L, Wattiez A, Suwaidi S, et al. Pathogenesis based diagnosis and treatment of endometriosis. Front Endocrinol (Lausanne). 2021;12:745548. https://doi.org/10.3389/fendo.2021.745548
3. Kiesel L, Sourouni M. Diagnosis of endometriosis in the 21st century. Climacteric. 2019;22(3):296-302. https://doi.org/10.1080/13697137.2019.1578743
4. Ahn SH, Singh V, Tayade C. Biomarkers in endometriosis: challenges and opportunities. Fertil Steril. 2017;107(3):523-32. https://doi.org/10.1016/j.fertnstert.2017.01.009

5. Parada LRC, Turri JAO, Helena Costa V, Vieira IB, Baracat EC, Soares Júnior JM, et al. Non-oncological gynecological diagnoses in a women's health care service during the pandemic caused by the severe acute respiratory syndrome coronavirus 2. PLoS One. 2023;18(3):e0282039. https://doi.org/10.1371/journal.pone.0282039
6. Jurczak P, Groves P, Szymanska A, Rodziewicz-Motowidlo S. Human cystatin C monomer, dimer, oligomer, and amyloid structures are related to health and disease. FEBS Lett. 2016;590(23):4192-201. https://doi.org/10.1002/1873-3468.12463
7. Shlipak MG, Inker LA, Coresh J. Serum cystatin C for estimation of GFR. JAMA. 2022;328(9):883-4. https://doi.org/10.1001/jama.2022.12407
8. Omaygenç MO, Özcan ÖU, Çakal B, Karaca O. Cystatin C and uncontrolled hypertension. Anatol J Cardiol. 2020;24(5):309-15. https://doi.org/10.14744/AnatolJCardiol.2020.78974
9. Peng M, Chen Y, Geng G. Cystatin C and intravenous thrombolysis. Eur J Neurol. 2021;28(4):e28. https://doi.org/10.1111/ene.14722
10. Gomes HCDS, Cabral ACV, Andrade SP, Leite HV, Teixeira PG, Campos PP, et al. Cystatin C as an indicator of renal damage in pre-eclampsia. Hypertens Pregnancy. 2020;39(3):308-13. https://doi.org/10.1080/10641955.2020.1766488
11. Ding L, Liu Z, Wang J. Role of cystatin C in urogenital malignancy. Front Endocrinol (Lausanne). 2022;13:1082871. https://doi.org/10.3389/fendo.2022.1082871
12. Bellos I, Fitrou G, Daskalakis G, Papantoniou N, Pergialiotis V. Serum cystatin-C as predictive factor of preeclampsia: a meta-analysis of 27 observational studies. Pregnancy Hypertens. 2019;16:16:97-04. https://doi.org/10.1016/j.preghy.2019.03.006
13. Yuan X, Han X, Jia C, Wang H, Yu B. Association of maternal serum uric acid and cystatin C levels in late pregnancy with adverse birth outcomes: an observational cohort study in China. Int J Womens Health. 2022;14:213-23. https://doi.org/10.2147/IJWH.S350847
14. Zhang H, Sun T. Correlation of blood glucose and pancreatic islet function with serum retinol-binding protein 4, serum cystatin C, and human new satiety molecule protein-1 in pregnant women with gestational diabetes mellitus. Evid Based Complement Alternat Med. 2022;2022:4247412. https://doi.org/10.1155/2022/4247412
15. Zhang L, Zhang L, Wang Z, Zhu L, Wang H, Chen H, et al. Increased risk markers in women with polycystic ovary syndrome and gestational diabetes mellitus during mid-pregnancy. J Int Med Res. 2020;48(8):300060520934633. https://doi.org/10.1177/0300060520934633
16. Yildirim A, Yildizhan B, Anik Ilhan G, Pekin T. Cystatin C, a novel cardiometabolic risk marker in women with polycystic ovary syndrome. Gynecol Endocrinol. 2016;32(6):457-9. https://doi.org/10.3109/09513590.2015.1130807
17. Ta□kömür AT, Erten Ö. Relationship of inflammatory and metabolic parameters in adolescents with PCOS: BMI matched case-control study. Arch Endocrinol Metab. 2022;66(3):372-81. https://doi.org/10.20945/2359-3997000000497
18. Çınar M, Aksoy RT, Güzel A□, Tokmak A, Çandar T, Ta□çı Y. The predictive role of serum cystatin C levels in polycystic ovary syndrome in adolescents. J Pediatr Adolesc Gynecol. 2016;29(4):353-6. https://doi.org/10.1016/j.jpag.2015.12.005
19. Coutinho LM, Ferreira MC, Rocha ALL, Carneiro MM, Reis FM. New biomarkers in endometriosis. Adv Clin Chem. 2019;89:89:59-77. https://doi.org/10.1016/bs.acc.2018.12.002
20. Socolov R, Socolov D, Sindilar A, Pavaleanu I. An update on the biological markers of endometriosis. Minerva Ginecol. 2017;69(5):462-7. https://doi.org/10.23736/S0026-4784.17.04046-1
21. Menzhinskaya IV, Melkumyan AG, Pavlovich SV, Chuprynin VD, Vanko LV, Sukhikh GT. [Autoimmune markers for non-invasive diagnosis of endometriosis in women]. Biomed Khim. 2020;66(2):162-6. https://doi.org/10.18097/PBMC20206602162
22. Lobo VL, Soares JM, Jesus Simões M, Simões RS, Lima GR, Baracat EC. Does gestrinone antagonize the effects of estrogen on endometrial implants upon the peritoneum of rats? Clinics (Sao Paulo). 2008;63(4):525-30. https://doi.org/10.1590/s1807-59322008000400019
23. Encalada Soto D, Rassier S, Green IC, Burnett T, Khan Z, Cope A. Endometriosis biomarkers of the disease: an update. Curr Opin Obstet Gynecol. 2022;34(4):210-9. https://doi.org/10.1097/GCO.0000000000000798
24. Fettback PB, Pereira RM, Rocha AM, Soares JM, Smith GD, Baracat EC, et al. Expression of stem cell-related genes in the endometrium and endometriotic lesions: a pilot study. Gynecol Endocrinol. 2016;32(1):82-6. https://doi.org/10.3109/09513590.2015.1092135
25. Moein Mahini S, Younesi M, Mortazavi G, Samare-Najaf M, Karim Azadbakht M, Jamali N. Non-invasive diagnosis of endometriosis: immunologic and genetic markers. Clin Chim Acta. 2023;538:70-86. https://doi.org/10.1016/j.cca.2022.11.013