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Red blood cell indices as an effective marker for the existence and severity of endometriosis (STROBE)

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Abstract

To evaluate the association between red blood cell (RBC) indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], MCH concentration [MCHC], red cell distribution width [RDW], hemoglobin [Hb], hematocrit [Hct], and neutrophil-to-lymphocyte ratio [NLR]) and the severity of endometriosis.

Data were obtained from the medical records of 200 patients with endometriosis (stage I/II and stage III/IV groups), and 100 patients with benign ovarian tumors (control group), treated between September 2011 and April 2021.

The mean Hb and Hct were significantly lower in the stage III/IV group compared to those in the control and stage I/II group ($P = .015$ and $P = .004$, respectively). The mean MCV, MCH, and Hb at postoperative day (POD)#1 were significantly lower in the stage III/IV group compared to those in the control and stage I/II group ($P = .007$, $P = .032$, and $P < .0001$, respectively). In addition, NLR at POD#1 was significantly higher in stage III/IV group compared to that in the control and stage I/II group. Multivariate analysis revealed that younger age (≤38 years old), lower preoperative MCV (≤88.5 fL), lower POD#1 Hb (<11.6g/dL), and higher POD#1 NLR (>2.5) were independent risk factors of stage III/IV endometriosis.

Lower levels of RBC indices, including preoperative MCV and postoperative Hb, and higher postoperative NLR were significantly associated with the severity of endometriosis, which is potentially derived from a dysregulation in iron metabolism and inflammation.

Abbreviations: Hb = hemoglobin, Hct = hematocrit, MCH = mean corpuscular hemoglobin, MCHC = MCH concentration, MCV = mean corpuscular volume, NLR = neutrophil-to-lymphocyte ratio, POD = postoperative, RBC = red blood cell, RDW = red cell distribution width, ROS = reactive oxygen species.

Keywords: endometriosis, mean corpuscular hemoglobin, mean corpuscular volume, RBC indices, red blood cell, red cell distribution width

1. Introduction

Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity. The estimated prevalence of endometriosis is 10 to 15% in the general population, and up to 70% in women with chronic pelvic pain.[1,2] Patients with endometriosis often experience dysmenorrhea and infertility, resulting in a severely limited quality of life.[2] Despite a large research effort, the etiology and pathophysiology of the disease have not been fully elucidated.[2]

However, it has recently been suggested that endometriosis has a connection with the complete blood cell count.[3–5] Endometriosis is a chronic inflammatory disease, therefore, various inflammatory markers, including lymphocytes, interleukin, and neutrophil-to-lymphocyte ratio (NLR) are elevated in severe endometriosis.[6–8]

Specifically, the NLR has been proposed as a simple and feasible diagnostic or prognostic indicator for various diseases.[3,6,9]

Furthermore, other evidence suggests that an increase in reactive oxygen species (ROS) formation induced by a high iron concentration is an early event in the development of endometriosis.[10]

In addition, several recent studies suggest that more severe endometriosis is associated with a lower hemoconcentration, along with dysregulation of red blood cells (RBC) or iron metabolism.[11,12]

In this study, we aimed to evaluate relationship among the RBC indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], MCH concentration [MCHC], red cell distribution width [RDW], hemoglobin [Hb], hematocrit blood test [Hct]), NLR, and endometriosis.

2. Materials and Methods

Retrospective data was collected from chart review of premenopausal women who underwent laparoscopic surgery for

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

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the treatment of benign ovarian tumor at the Department of Obstetrics and Gynecology, Kangnam Sacred Heart Hospital, between September 2011 and April 2021. The study received approval from the institutional review board (**IRB no. 2017-02-13**). Women who were pathologically confirmed or clinically suspected of pregnancy, leiomyoma, adenomyosis, pelvic inflammatory disease, tuberculosis, hematemesis, endocrine disease, abnormal liver function, heart disease, diabetes, kidney disease, cancer, or an immune system disease were excluded. In addition, women who underwent combined surgery, such as laparoscopic myomectomy and laparoscopic adenomyomectomy were excluded.

Total 300 women were included in this study, of which 200 were pathologically confirmed with endometriosis (study group) and other 100 were pathologically confirmed with other benign adnexal diseases (control group), such as teratoma, serous cystadenoma, and mucinous cystadenoma. Among the 200 patients with endometriosis, 100 women had stage I to II endometriosis (stage I to II group) and 100 women had stage III to IV endometriosis (stage III to IV group). All patients underwent routine preoperative laboratory studies, including a complete blood cell count performed within one month before the surgery. RBC indices and NLR were calculated as follows:

1. Red cell distribution width [RDW]: (Standard deviation of MCV/MCV) × 100.
2. Mean corpuscular volume [MCV]: Hct (%) × 10/RBC count (million/μL).
3. Mean corpuscular hemoglobin [MCH]: Hb (g/dL) × 10/RBC count (million/μL).
4. Mean corpuscular hemoglobin concentration [MCHC]: Hb (g/dL) × 100/Hct (%).
5. Hemoblogin [Hb]: MCV × RBC/29.8.
6. Hematocrit [Hct]: (RBC × MCV)/10.
7. Neutrophil to lymphocyte ratio [NLR]: neutrophil count/lymphocyte count.

Symptoms including dysmenorrhea, heavy menstrual bleeding, and dyspareunia before surgery were recorded; however, the menstrual cycle of each patient was not considered. The endometriosis score was determined using the revised American Society for Reproductive Medicine guidelines.[13]

Statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL) and Medcalc software (version 15.2.2, Portland, USA). *P* values <.05 were considered statistically significant. Group differences were evaluated using one-way analysis of variance (ANOVAs), with post hoc comparisons assessed using Duncan Multiple Range test. Multivariate analysis was performed using binary logistic regression. The hazard ratio (HR) and 95% confidence intervals (CI) were calculated. The accuracy of RBC indices and NLR in discrimination of severe endometriosis (stage III–IV) from mild endometriois using the receiver operating characteristics (ROC) curve analysis.

3. Results

The basic patient characteristics are summarized in Table 1, and the symptoms are summarized in Table 2. There were no significant group differences in the mean body weight or body mass index (BMI). Many of the women with endometriosis suffered from dysmenorrhea and heavy menstrual bleeding. Table 3 provides the mean values for RBC, Hb, Hct, MCV, MCH, MCHC, RDW, and postoperative day 1 Hb. Significant group differences were observed for Hb, Hct, MCV, MCH, and Hb at postoperative day (POD)#1 (*P* = .015, .004, .007, .032, <.0001, respectively). These RBC indices were significantly lower in the stage III to IV group compared to those in the control and stage I to II groups. However, there were no significant group differences in RBC count, MCHC, or RDW.

Mean NLR and platelet counts are also shown in Table 3. Significant group differences were observed in the mean NLR at POD#1 (*P* < .0001), with higher values in the stage III to IV group compared to those in the control and stage I to II groups. There were no significant group differences in the preoperative NLR, NLR at POD#3, or platelet count.

Multiple logistic regression analysis revealed that younger age (≤38 years old), lower preoperative MCV (≤88.5 fL), lower POD#1 Hb (<11.6g/dL), and higher POD#1 NLR (>2.5) were independent risk factors of stage III/IV endometriosis (Table 4).

4. Discussion

Endometriosis is related to local and systemic inflammatory processes. The peritoneal microenvironment in women with endometriosis includes an abundance of inflammatory cytokines, chemokines, and prostaglandins. Inflammation is also present in the ectopic endometrium of women with endometriosis.[14] Numerous studies have demonstrated that diverse inflammatory markers are found in the serum and peritoneal fluid of patients with endometriosis. A study evaluating diagnostic value of NLR in endometriosis revealed that NLR

Table 2

Patients' symptoms.

	Control (N = 100)	Stage I–II (N = 100)	Stage III–IV (N = 100)	Total (N = 300)
None	74	20	19	113
Dysmenorrhea	6	64	61	131
Heavy menstrual bleeding	0	26	22	48
Dyspareunia	1	0	0	1
Others*	19	51	43	113

Data are presented as number.

*Symptoms includes infertility, lower abdominal pain, and back pain.

Table 1

Group differences in basic patients' characteristics.

Characteristics	Control (N = 100)	Stage I–II (N = 100)	Stage III–IV (N = 100)	*P* value
Age(yr)	41.830 (39.475–44.184)	32.500 (30.915–34.084)	30.990 (29.613–32.366)	<.0001**
Height(cm)	160.159 (159.143–161.175)	160.173 (159.075–161.270)	161.980 (160.888–163.071)	.024*
Body weight(kg)	56.732 (55.189–58.274)	55.308 (53.564–57.052)	55.708 (53.822–57.594)	.493
BMI(kg/m^2)	22.138 (21.538–22.739)	21.521 (20.942–22.099)	21.232 (20.548–21.917)	.115
Hospital stay(days)	5.060 (4.896–5.224)	4.500 (4.245–4.754)	5.560 (5.113–6.006)	<.0001**

Note: Data are presented as mean (95% confidence interval), *P* values obtained by One-way analysis of variance.

BMI = body mass index.

*$P < .05$.

**$P < .0001$.

Table 3

RBC indices, neutrophil-to-lymphocyte ratio (NLR), and platelet count in each group.

	Control (N = 100)	Stage I–II (N = 100)	Stage III–IV (N = 100)	*P* value
Preoperative RBC indices				
RBCs (×10^6/uL)	4.302 (4.242–4.362)	4.259 (4.197–4.322)	4.264 (4.204–4.323)	.560
Hb (g/dL)	12.888 (12.668–13.107)	12.735 (12.551–12.918)	12.471 (12.267–12.674)	.015*
Hct (%)	38.101 (37.553–38.678)	37.536 (36.998–38.073)	36.806 (36.266–37.345)	.004*
MCV (fL)	88.646 (87.521–89.770)	88.243 (87.326–89.159)	86.514 (85.581–87.447)	.007*
MCH (pg)	30.039 (29.557–30.520)	29.950 (29.589–30.310)	29.312 (28.899–29.725)	.032*
MCHC (%)	33.848 (33.626–34.069)	33.940 (33.734–34.145)	33.860 (33.631–34.088)	.814
RDW (%)	12.786 (12.548–13.023)	12.698 (12.468–12.928)	12.767 (12.559–12.974)	.850
POD#1 Hb (g/dL)	11.778 (11.556–11.999)	11.723 (11.519–11.926)	11.110 (10.903–11.316)	<.0001**
Preoperative NLR	2.025 (1.768–2.283)	2.841 (1.883–3.798)	2.539 (1.928–3.150)	.229
POD#1 NLR	3.106 (2.730–3.481)	3.150 (2.702–3.597)	4.480 (3.833–5.126)	<.0001**
POD#3 NLR	2.645 (1.785–3.505)	2.334 (1.996–2.671)	2.519 (2.207–2.831)	.738
Platelet (×10^3/uL)	264.18 (252.93–275.43)	258.03 (246.33–269.73)	256.93 (245.50–268.42)	.635

Note: Data are presented as mean (95% confidence interval), *P* values obtained by One-way analysis of variance.

Hb = hemoglobin, Hct = hematocrit blood test, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, NLR = neutrophil-to-lymphocyte ratio, POD = postoperative day, RBC = red blood cell, RDW = red cell distribution width.

* $P < .05$.

** $P < .0001$.

Table 4

Risk factors of severe endometriosis.

Risk factors	HR (95% CI)	*P* value
Age (≤38 yrs old)	3.936 (1.961–7.898)	<.0001**
Preoperative Hb (≤13.0 g/dL)	0.744 (0.310–1.784)	.507
Preoperative Hct (≤37.9%)	2.028 (0.892–4.611)	.091
Preoperative MCV (≤88.5 fL)	2.339 (1.131–4.837)	.022*
Preoperative MCH (≤29.5 pg)	0.830 (0.395–1.744)	.622
POD#1 Hb (<11.6g/dL)	2.331 (1.211–4.484)	.011*
POD#1 NLR (>2.5)	3.065 (1.725–5.447)	<.0001**

Hb = hemoglobin, Hct = hematocrit blood test, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, NLR = neutrophil-to-lymphocyte ratio, POD = postoperative day.

* $P < .05$.

** $P < .0001$.

shows a lower sensitivity of 57.9% and specificity of 65.2% with a cutoff value at 1.82. Furthermore, the combination of NLR and CA125 has the highest AUC of 0.949 with a sensitivity of 86.8% and specificity of 92.0% at the cutoff value of 44.40.[5]

According to Sampson's theory, retrograde menstruation is considered as an essential process in the pathogenesis of peritoneal endometriosis.[15] Retrograde menstruation transports menstrual endometrial tissue and RBCs through the fallopian tubes into the peritoneal cavity. In fact, RBCs are present in the peritoneal fluid of most women. A review article reported that 21 studies have demonstrated the presence of iron overload in the different components of the peritoneal cavity (peritoneal fluid, ectopic endometrial tissue, the peritoneum adjacent to lesions, and macrophages).[16] In addition, a study suggested that iron overload by retrograde menstruation induces oxidative stress, which may permit ectopic survival of endometrial cells and lesion vascularization in the peritoneal cavity.[17] Only a few studies have shown that RBC indices have a negative correlation with endometriosis. Kim et al reported that more severe endometriosis is associated with a lower hemoconcentration.[3]

In our study, the mean values of preoperative and postoperative day 1 Hb, Hct, MCV, and MCH were found to be significantly negatively correlated with the existence of endometriosis as well as the endometriosis stage. Thus, these RBC indices appear to be related to endometriosis severity. We suggest that these findings result from a dysregulation in iron metabolism. In humans, the body iron content is ~45 mg Fe/kg body weight, with typically higher values in men than in women. Circulating RBCs contain most of this iron, which is bound to the oxygen transport protein, Hb (~30 mg Fe/kg).[18] Increased concentrations of erythrocytes have been reported in the peritoneal cavity of women with endometriosis.[1] In the case of endometriosis, iron overload may originate from the lysis of pelvic erythrocytes.[19] Iron overload in the pelvic cavity can affect numerous mechanisms involved in the development of endometriosis.[20] Thus, the observed negative correlation between endometriosis severity and specific RBC indices may be derived from a dysregulation in iron metabolism.

However, there was no association between other RBC indices (RBC count, MCH, MCHC, and RDW) and the endometriosis stage. Thus, the pathophysiology of endometriosis requires further evaluation through large randomized controlled trials.

As mentioned above, a number of studies have suggested that severe endometriosis is correlated with NLR elevation.[21–23] A retrospective study including 662 women who had undergone laparoscopic surgery for the treatment of endometriosis revealed that lower NLR was significantly associated with infertility.[21] Also, NLR was significantly higher in patients with endometriotic cysts of >5 cm diameter or oviduct adhesions ($P = .008$, $P = .048$, respectively).[21] The authors suggested that NLR may be used as a simple and easily obtained predictive marker for endometriosis with infertility.[21] On the contrary, in our study, only the mean NLR at postoperative day 1 was found to be positively correlated with the existence of endometriosis as well as the endometriosis stage; the preoperative and postoperative day 3 NLRs were not related to endometriosis existence or severity. Therefore, additional large-scale studies are needed to clarify the relationship between NLR and endometriosis.

The strengths of our study include the selection of controls. We included only women who had undergone surgery for an ovarian benign tumor, allowing confirmation that the control subjects did not have endometriosis. The primary limitation of our study concerns its retrospective design. Menstrual cycles can affect the results of complete blood count test. However, we performed blood sampling irrespective of patients' menstrual cycles, which might be a potential source of bias. In addition, anemia is very common in premenopausal women. Although the exclusion criteria were strictly observed, we could not exclude other hidden causes of anemia.

In conclusion, the present findings demonstrate that lower RBC indices, such as preoperative MCV and POD#1 Hb, and higher POD#1 NLR were significantly associated with stage III/IV endometriosis. We consider that lower RBC indices might be

derived from dysregulation of iron metabolism. Also, a higher NLR might be stem from inflammation associated with severe endometriosis. However, the pathophysiology of our findings remains unclear and further multicenter prospective studies are needed to evaluate these findings.

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Author contributions

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References

[1] Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789–99.
[2] Parasar P, Ozcan P, Terry KL. Endometriosis: epidemiology, diagnosis and clinical management. Curr Obstet Gynecol Rep. 2017;6:34–41.
[3] Kim SK, Park JY, Jee BC, et al. Association of the neutrophil-to-lymphocyte ratio and CA 125 with the endometriosis score. Clin Exp Reprod Med. 2014;41:151–7.
[4] Yang H, Zhu L, Wang S, et al. Noninvasive diagnosis of moderate to severe endometriosis: the platelet-lymphocyte ratio cannot be a neoadjuvant biomarker for serum cancer antigen 125. J Minim Invasive Gynecol. 2015;22:373–7.
[5] Yang H, Lang JH, Zhu L, et al. Diagnostic value of the neutrophil-to-lymphocyte ratio and the combination of serum CA-125 for stages III and IV endometriosis. Chin Med J (Engl). 2013;126:2011–4.
[6] Tokmak A, Yildirim G, Oztas E, et al. Use of neutrophil-to-lymphocyte ratio combined with CA-125 to distinguish endometriomas from other benign ovarian cysts. Reprod Sci. 2016;23:795–802.
[7] Jiang L, Yan Y, Liu Z, et al. Inflammation and endometriosis. Front Biosci (Landmark Ed). 2016;21:941–8.
[8] Kokot I, Piwowar A, Jedryka M, et al. Diagnostic significance of selected serum inflammatory markers in women with advanced endometriosis. Int J Mol Sci . 2021;22.
[9] Hirahara T, Arigami T, Yanagita S, et al. Combined neutrophil-lymphocyte ratio and platelet-lymphocyte ratio predicts chemotherapy response and prognosis in patients with advanced gastric cancer. BMC Cancer. 2019;19:672.
[10] Donnez J, Binda MM, Donnez O, et al. Oxidative stress in the pelvic cavity and its role in the pathogenesis of endometriosis. Fertil Steril. 2016;106:1011–7.
[11] Atkins HM, Appt SE, Taylor RN, et al. Systemic iron deficiency in a non-human primate model of endometriosis. Comp Med. 2018;68:298–307.
[12] Manolov V, Marinov B, Vasilev V. [Serum hepcidin levels in endometriosis]. Akush Ginekol (Sofiia). 2015;54(Suppl 1):32–8.
[13] Sillem M, Prifti S, Schmidt M, et al. Endometrial integrin expression is independent of estrogen or progestin treatment in vitro. Fertil Steril. 1997;67:877–82.
[14] Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril. 2012;98:511–9.
[15] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol. 1927;3:93–110.43.
[16] Defrere S, Lousse JC, Gonzalez-Ramos R, et al. Potential involvement of iron in the pathogenesis of peritoneal endometriosis. Mol Hum Reprod. 2008;14:377–85.
[17] Pirdel L, Pirdel M. Role of iron overload-induced macrophage apoptosis in the pathogenesis of peritoneal endometriosis. Reproduction. 2014;147:R199–207.
[18] Harrison-Findik DD. Role of alcohol in the regulation of iron metabolism. World J Gastroenterol. 2007;13:4925–30.
[19] Van Langendonckt A, Casanas-Roux F, Eggermont J, et al. Characterization of iron deposition in endometriotic lesions induced in the nude mouse model. Hum Reprod. 2004;19:1265–71.
[20] Defrere S, Gonzalez-Ramos R, Lousse JC, et al. Insights into iron and nuclear factor-kappa B (NF-kappaB) involvement in chronic inflammatory processes in peritoneal endometriosis. Histol Histopathol. 2011;26:1083–92.
[21] Jing X, Li C, Sun J, et al. Systemic inflammatory response markers associated with infertility and endometrioma or uterine leiomyoma in endometriosis. Ther Clin Risk Manag. 2020;16:403–12.