RESEARCH ARTICLE

Are Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios Associated with Endometrial Precancerous and Cancerous Lesions in Patients with Abnormal Uterine Bleeding?

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Abstract

Background: An easy, reproducible and simple marker is needed to estimate phase of endometrial pathologic lesions such as hyperplasia and endometrial cancer and distinguish from pathologically normal results. We here aimed to clarify associations among neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), endometrial hyperplasia and cancer in patients with abnormal uterine bleeding. Materials and Methods: Patients (n=161) who were admitted with abnormal uterine bleeding and the presence of endometrial cells on cervical cytology or thick endometrium were investigated. The study constituted of three groups according to pathologic diagnosis. Group 1 included endometrial precancerous lesions like hyperplasia (n=63), group 2 included endometrial cancerous lesions (n=38) and group 3 was a pathologically normal group (n=60). Blood samples were obtained just before the curettage procedure and the NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count; similarly, PLR was defined as the absolute platelet count divided by the absolute lymphocyte count. Results: The white blood cell count was significantly higher in patients with cancer than in those with hyperplasia (p=0.005). The platelet count and neutrophil to lymphocyte ratio were significantly higher in patients with cancer than in control patients, but there was significantly no difference between patients with hyperplasia and other groups (p=0.001 and p=0.025 respectively). PLR was significantly lower in control subjects than in other groups (p<0.001), but there was no significant difference between patients with hyperplasia and those with cancer. Conclusions: PLR was significantly lower in control subjects than in other groups. Thus both hyperplasia and cancer may be differentiated from pathologically normal patients by using PLR. White blood cell count was significantly higher in patients with cancer than in those with hyperplasia and pathologically normal patients. Therefore white blood cell count may be used for discriminate hyperplasia to cancer. By using multiple inflammation parameters, discrimination may be possible among endometrial cancer, endometrial precancerous lesions and pathologically normal patients.

Keywords: Endometrial cancer - endometrial hyperplasia - neutrophil to lymphocyte ratio - platelet to lymphocyte ratio.

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Introduction

Inflammation is a well known feature of cancer and plays an important role in various aspects of cancer involving cancer initiation, promotion, progression, metastasis and clinical features (Babu et al., 2012). Numbers of researchers have investigated possible associations between chronic inflammation and cancer, whereas the precise mechanisms remain uncertain. Current knowledge suggests a reciprocal induction between chronic inflammation and cancer (Guo et al., 2013). Endometrial hyperplasia (EH), which is thought to be caused by the prolonged, unopposed estrogenic stimulation of the endometrium, is a known risk factor for the development of endometrial cancer, particularly atypical EH, with a subsequent risk of up to 30% (Heller et al., 2011). Adenocarcinoma of the endometrium is the most common gynecological cancer (Turan et al., 2012). Association of inflammation and various cancers were investigated by some authors. It is established that the neutrophil-to-lymphocyte ratio (NLR) is one of the nonspecific marker of inflammation. A high blood neutrophil-to-lymphocyte ratio has been shown to be associated with poor survival in patients with malignant mesothelioma and with disease recurrence in esophageal cancer (Kao et al 2010; Sharaiha et al., 2011). Some markers of systemic inflammation such as C-reactive protein (CRP) have been correlated with outcomes in over carcinoma and renal cell carcinoma

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(Saito et al., 2009; Toriola et al., 2011). It has also been reported that the platelet-to-lymphocyte ratio (PLR) was a significant prognostic factor in operable colorectal cancer (Kwon et al., 2012). Thus, an easily reproducible and simple marker is needed to estimate phase of endometrial pathologic lesions such as hyperplasia, endometrial cancer or pathologically normal results. To the best of our knowledge, there have been no previous reports about the association among NLR, PLR, endometrial hyperplasia and cancer. We aimed to investigate association of NLR, PLR, endometrial hyperplasia and cancer in patients with abnormal uterine bleeding.

Materials and Methods

A retrospective cross-sectional study was conducted at the Kayseri Education and Research Hospital, Department of Obstetrics and Gynecology, Kayseri, Turkey, between May 2013 and November 2013. During a 6-month period all women (n=180) who were referred for abnormal uterine bleeding in postmenopausal and premenopausal period or the presence of endometrial cells on cervical cytology to the our department, were included to participate in this study, and women ages 35-57 years, who fulfilled the inclusion criteria, were selected for inclusion. The study was approved by the institutional review board of Kayseri Education and Research Hospital of Medicine.

A complete physical and gynecological examination was performed and routine laboratory tests were obtained to exclude systemic causes of bleeding. All patients underwent transvaginal ultrasound scanning to exclude the presence of other pathologies and to assess the endometrial thickness. Venous blood (10cc) was obtained from antecubital vein for routine blood examination such as human chorionic gonadotropine, blood count and prothrombin time, activated prothrombin time and international normalized ratio (INR)… etc just before curettage procedure. After test results were obtained, all patients had undergone curettage procedure.

The inclusion criteria were, patients who had symptoms of abnormal bleeding or the presence of endometrial cells on cervical cytology among women at postmenopausal or premenopausal age. Moreover patients who were administered our menopause clinic for routine control with thick endometrium >4mm in postmenopausal period, were included into the study. Nineteen women were excluded because of the presence of at least one of the exclusion criteria: use of steroid hormones during the 12 months prior to the study or use of oral contraceptives during their lifetime, patients with family history of endometrial cancer, liver disease, tamoxifen use, ovarian or endometrial tumor, endometriosis, patients with second malignancies, hematological disease, inflammatory disease, recombinant granulocyte colony-stimulating factor use, or missing preoperative complete blood cell count or complete blood cell count drawn more than two weeks prior to surgery, bilateral oophorectomy or previous hysterectomy or endometrial ablation. The cases in which material was insufficient for biopsy were not considered in the analysis. At least we were capable of investigating 161 volunteers with abnormal uterine bleeding and the presence of endometrial cells on cervical cytology or thick endometrium.

Gynecologic pathologists assessed the histological samples and classified them using the 1994 World Health Organization classification of EH. This classification is comprised of four categories: (1) simple EH without atypia, (2) complex EH without atypia, (3) simple atypical EH and (4) complex atypical EH (Kleebkaow et al., 2008). We classified the findings as precancerous lesions (EH) and normal results (secretuar endometrium, atrophic endometrium, proliferative endometrium and endometrial cells) and endometrial cancer then for the purpose of study women were divided into three groups as group 1 (women with precancerous lesions group n=63), group 2 (endometrial cancer group n=38) and group 3 (pathologically normal group n=60) then we made comparisons among these three groups.

Diagnosis was based on outpatient endometrial curettage material. Dilatation and curettage was performed to according to our clinics description.

Blood samples were obtained just before curettage procedure and the NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count; similarly, PLR was defined as the absolute platelet count divided by the absolute lymphocyte count.

Statistical analysis

SPSS 15.0 statistic software was used for the statistical analysis. The Kolmogorov-Smirnov test was used to determine the normality of distributions of variables. Continuous variables with normal distribution were presented as mean±standard deviation. Median value (minimum-maximum) was used in variables without normal distribution. Statistical analysis for the parametric variables was performed by one-way ANOVA with Scheffe's post-hoc test among three groups. The Kruskal-Wallis test was used to compare the nonparametric variables. Then, the Mann-Whitney U-test with Bonferroni correction was used to assess differences among three groups. $p < 0.05$ was considered to be significant.

Results

Mean age was 46.6±8.7 years. Median neutrophil to lymphocyte ratio and platelet to lymphocyte ratio were 2.09 (0.61-18.40) and 118.3 (45.6-743.2), respectively. Table 1 shows the blood characteristics of 161 patients.

The comparison of age and blood parameters among the three groups is shown in Table 2. Age was significantly higher in patients with cancer than in other groups, but

Table 1. The Characteristics of 161 Patients

Characteristic	
Age (year)	46.6±8.7
Hemoglobin (g/dL)	12.1±2.0
Hemotocrit (%)	37.5±5.2
White blood cell count (/μL)	8107±2535
Platelet count (/μL)	289447±96910
Neutrophil to lymphocyte ratio	2.09 (0.61-18.40)
Platelet to lymphocyte ratio	118.3 (45.6-743.2)

Table 2. Comparison of Age and Blood Parameters among the three Groups

	Patients with hyperplasia (n:63)	Patients with cancer (n:38)	Control patients (n:60)	p value
Age (year)[a]	45.4±6.4	53.2±11.7	43.8±6.1	<0.001
Hemoglobin (g/dL)	11.9±2.1	12.4±1.6	12.2±2.0	0.413
Hemotocrit (%)	37.6±5.9	37.7±4.4	37.3±5.0	0.930
White blood cell count (/μL)[b]	7488±1775	9171±3104	8083±2635	0.005
Platelet count (/μL)[c]	294064±73930	331605±142345	257900±69562	0.001
Neutrophil to lymphocyte ratio[c]	2.01 (0.95-4.69)	2.89 (0.98-18.40)	1.94 (0.61-4.89)	0.025
Platelet to lymphocyte ratio[d]	121.2 (58.5-400.0)	144.9 (70.2-743.2)	103.7 (45.6-227.7)	<0.001

[a]Age was significantly higher in patients with cancer than in other groups; [b]White blood cell count was significantly higher in patients with cancer than in those with hyperplasia; [c]Platelet count and neutrophil to lymphocyte ratio were significantly higher in patients with cancer than in control patients; [d]Platelet to lymphocyte ratio was significantly lower in control subjects than in other groups

there was no significant difference between patients with hyperplasia and control subjects. White blood cell count was significantly higher in patients with cancer than in those with hyperplasia, but there was significantly no difference between control subjects and other groups. Platelet count and *neutrophil* to *lymphocyte ratio* were significantly higher in patients with cancer than in control patients, but there was significantly no difference between patients with hyperplasia and other groups. *Platelet to lymphocyte ratio was significantly lower in control subjects than in other groups, but* there was significantly no difference between patients with hyperplasia and those with cancer. There was significantly no difference among the three groups in terms of hemoglobin concentration and hemotocrit level ($p > 0.05$).

Discussion

In a recent study authors investigated blood count in patients with malignant tumors. They found that patients with cancer have an increased CRP level, the presence of neutrophilia and relative lymphocytopenia secondary to the systemic inflammatory response. They concluded that tumor-associated neutrophils (TANS) promote remolding of the extracellular matrix via their enzyme action, which results in the release of basic fibroblast growth factor, migration of endothelial cells and the dislocation of tumor cells. These events finally result in enhanced angiogenesis, and tumor growth and progression to a metastatic phenotype (Fridlender et al., 2009). This finding is supported by other trials and neutrophils have been associated with poor survival in patients with metastatic renal cell carcinoma and advanced non-small cell lung cancer (Négrier et al., 2002; Teramukai et al., 2009). Other researchers found out that neutrophilia was more frequently associated with recurrence and metastasis in patients with advanced-stage cervical cancer relative to preinvasive neoplasia and neutrophilia, and was the parameter that best predicted diseases progression (Tavares-Murta et al., 2010). Additionally it has been showed that patients with locally advanced cervical cancer who were treated with chemoradiation showed a complete response to treatment with a greater baseline lymphocyte count (Choi et al., 2008). Opposite of the above mentioned studies, authors examined the effect of treatment on lymphocyte count in cervical cancer patients. They concluded that baseline lymphocyte count was not a significant predictor of tumor response and progression-free survival in patients treated with neoadjuvant chemotherapy followed by surgery (Choi et al., 2006). In the present study our findings were compatible with other researchers however in literature patients with precancerous lesions did not have higher platelet to lymphocyte ratio than control group. This situation may be linked to different types of cancers involved in above mentioned studies. Among these studies, authors evaluated only cervical precancerous lesions in gynecologic oncology area however we evaluated endometrial precancerous lesions. In a study authors investigated PLR association with treatment response and prognosis. They concluded that pretreatment PLR can provide important prognostic results in patients with non-small cell lung cancer (Unal et al., 2013). The present study demonstrated that platelet to lymphocyte ratio was significantly lower in control subjects than in hyperplasia and cancer groups. Therefore PLR seems the best choice for distinguishing between precancerous lesions and pathologically normal groups. Lymphocytopenia has previously been shown to be associated with pancreatic adenocarcinoma and colorectal cancer (Fukunaga et al., 2004; Kishi et al., 2009). A reduced number of tumor-infiltrating lymphocytes in resected pancreatic adenocarcinoma specimens have also been found to be associated with poorer survival rates after surgery (Fukunaga et al., 2004). The NLR is assumed a simple and effective marker of inflammation (Wang et al., 2011). Thus NLR has been investigated for to show the association between NLR and poor prognosis in different kinds of cancer. (Fukunaga et al., 2004; Teramukai et al., 2009). Several possible factors may explain the relationship between high NLR and a worse prognosis in cancer patients. First, the immune response of the host to cancer is lymphocyte dependent. In additional, neutrophils contain and secrete the vast majority of circulating vascular endothelial growth factor that may play important roles in the progression of cancer (Kusumanto et al., 2003). We found out that gradually increasing NLR in patients with pathologically normal group, hyperplasia and cancer (1.94, 2.01, 2.89 respectively). There was significantly difference between cancer and pathologically normal groups. However there was no significant difference between hyperplasia and pathologically normal groups. Therefore NLR can be used for discrimination cancer from pathologically normal patients. The platelet count is an additional index of systemic inflammation elicited by the tumor. A number of proinflammatory cytokines such as IL-1 and IL-6 promote

megakaryocyte proliferation resulting in thrombocytosis (Klinger and Jelkmann, 2002; Alexandrakis et al., 2003). Thrombocytosis and lymphocyto- penia have both been correlated with the degree of host systemic inflammation, and the platelet-lymphocyte ratio reflects a novel marker incorporating both hematologic factors (Smith et al., 2008). We illustrated that platelet count was significantly high in patients with endometrial cancer than pathologically normal group. Thus platelet count may be useful for discrimination of cancer from pathologically normal patients. As far as we know, there have been no previous studies that have investigated these parameters in endometrial hyperplasia and endometrial cancer. We found out that PLR was significantly lower in control subjects than in other groups. Thus both hyperplasia and cancer may be differentiated from pathologically normal patients by using PLR. White blood cell count was significantly higher in patients with cancer than in those with hyperplasia. Therefore white blood cell count may be used for discriminate hyperplasia to cancer. Moreover platelet count and neutrophil to lymphocyte ratio were significantly higher in patients with cancer than in control patients. We are of the opinion that both parameters can be used for discrimination to cancer from pathologically normal patients. By using multiple inflammation parameters, discrimination may be possible among endometrial cancer, endometrial precancerous lesions and pathologically normal patients.

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