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Original Article

The significance of the neutrophil-to-lymphocyte ratio in differantial diagnosis of ectopic pregnancy and miscarriage

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Abstract: Objective: The aim of this study is to assess the diagnostic value of the neutrophil-to-lymphocyte ratio in the differential diagnosis of patients with vaginal bleeding related to ectopic pregnancy or miscarriage. Study design. A retrospective analysis of case histories of patients with documented ectopic pregnancies or miscarriages diagnosed by a combination of clinical examination, transvaginal ultrasonography, and human chorionic gonadotropin assay. Normal pregnant women were used as controls. The neutrophil-to-lymphocyte ratio was calculated in the patients and controls. Results: The neutrophil-to-lymphocyte ratio was higher in patients with ectopic pregnancies than in patients with normal pregnancies or patients with miscarriages ($P<0.05$). Although the neutrophil-to-lymphocyte ratio in patients with miscarriages was higher than that in patients with normal pregnancies, the difference was not statistically significant. For ectopic pregnancy, using 3.0 as a cutoff value, the sensitivity and specificity of the neutrophil-to-lymphocyte ratio were 61%. For miscarriage, using 2.8 as a cutoff value, the sensitivity and specificity of neutrophil-to-lymphocyte ratio were 53%. Conclusions: In patients with vaginal bleeding related to ectopic pregnancy or miscarriage, an increased neutrophil-to-lymphocyte ratio in the absence of infection may be used as an early diagnostic marker for ectopic pregnancy. Miscarriage did not result in asignificant increase in the neutrophil-to-lymphocyte ratio. Further studies performed in patients grouped by gestational age and rupture status are needed to determine the diagnostic value of the neutrophil-to-lymphocyte ratio in patients suspected of an ectopic pregnancy.

Keywords: Ectopic pregnancy, miscarriage, neutrophil-to-lymphocyte ratio

Introduction

Vaginal bleeding is commonly experienced (20-40%) by pregnant women in the first trimester and is related especially to ectopic pregnancy and miscarriage. The overall incidence of ectopic pregnancy is approximately 2% of reported pregnancies [1, 2]. Hemorrhage from ectopic pregnancy has been shown to be the leading cause of maternal mortality in the first trimester and accounts for 4-10% of all pregnancy-related deaths [2]. Miscarriage is the most common complication of early pregnancy and occurs with decreasing frequency with increasing gestational age. In the literature, its incidence in clinical pregnancies varies from 8% to 20% during the first half of pregnancy [3].

Early pregnancy complications can be very upsetting. Ectopic pregnancy and miscarriage have an adverse effect on the quality of a woman's life. They can cause considerable distress during the course of differential diagnosis and management. In patients with early pregnancy loss, there is a need to improve the reliability of diagnostic tests to allow prompt commencement of management options. These attempts can reduce the psychological burden of early pregnancy loss and the morbidity and mortality associated with ectopic pregnancy [4].

Although transvaginal ultrasonography and serum human chorionic gonadotropin (hCG) assays after pelvic examination are generally performed in the initial evaluation of patients with early pregnancy bleeding, the differential diagnosis of ectopic pregnancy and miscarriage may be challenging and can take one to two weeks. Several biomarkers have been studied to date in an attempt to ease the prompt diag-

Table 1. Selected demographic and clinical data of patients with ectopic pregnancy, those with miscarriage, and those with normal pregnancy

	Normal pregnancy (n = 153)	Ectopic pregnancy (n = 152)	Miscarriage (n = 80)
Age (y)	30.4 ± 4.9	30.7 ± 5.3	29.3± 7.3
BMI (kg/m^2)	25.1 ± 3.7	25.4 ± 2.8	25.7 ± 5.1
Gestational age (wk)	5.8 (4-12)	6.6 (4-9)[b]	7.5 (5-11)[a]
Pregnancy history			
Gravidity (number)	2 (1-8)	3 (1-15)[c]	3 (1-8)[d]
Parity	1 (0-3)[e]	2 (0-6)	1 (0-5)
Abortion	0 (0-5)	1 (0-15)	0 (0-6)
Dilatation & Curettage	0 (0-2)	0 (0-6)	0 (0-1)
Ectopic pregnancy	0 (0-1)	1 (0-2)[f]	0 (0-0)
History of cigarette smoking	1 (0.7%)[g]	19 (12.5%)	17 (21.3%)
History of intrauterine device use	0 (0%)	8 (5.3%)[h]	0 (0%)

Data were presented as mean ± SD, median (min-max) or percentage as appropriate. BMI, body mass index. [a]P<0.05 vs. ectopic pregnancy and normal pregnancy. [b,d]P<0.05 vs. normal pregnancy. [c,f,h]P<0.05 vs. miscarriage and normal pregnancy. [e,g]P<0.05 vs. miscarriage and ectopic pregnancy.

nosis of ectopic pregnancy [5-7]; However, none of the studied biomarkers has been deemed acceptable for clinical use. None of these previous studies assessed the neutrophil-to-lymphocyte ratio (NLR)-calculated as the neutrophil count divided by the lymphocyte count-as a possible biomarker for the differential diagnosis of ectopic pregnancy and miscarriage, although there were many studies that evaluated the clinical value of the NLR as a sensitive marker of inflammation and a possible prognostic indicator in several acute or chronic disorders including preeclampsia, adnexal torsion, Prognostic significance of the neutrophil to lymphocyte ratio in patients with non-small cell lung cancer, ovarian, or colorectal cancer [8-13]. Buyukkaya et al. [14] showed that there was a significant correlation between the NLR and the criteria for metabolic syndrome and inflammation. The NLR is also accepted as a marker for general immune responses to various stress stimuli [15]. We think that the NLR may be helpful in the differential diagnosis of ectopic pregnancy and miscarriage, because the pathogenesis of both conditions involves inflammatory and immunological processes. The aim of the current study was to investigate the value of the NLR as a differential diagnostic indicator in patients with vaginal bleeding related to ectopic pregnancy or miscarriage.

Materials and methods

A retrospective analysis of case histories of patients with documented ectopic pregnancy or miscarriage and who were admitted to our hospital from January 2010 to December 2014 was carried out. The study protocol was approved by the Human Research Ethics Committee of our university. The initial diagnosis of ectopic pregnancy or miscarriage, including gestational age, was made through a combination of clinical examination, transvaginal ultrasonography, and hCG assay. All patients suspected of having an ectopic pregnancy underwent a diagnostic laparoscopy. During the study period, normal pregnant women who attended the antenatal outpatient service and matched for maternal age were used as controls. Women in the first trimester of pregnancy were included. The exclusion criteria were: (a) the existence of other factors that may affect the proportion of white blood cells or may affect inflammation (smoking, fever, corticosteroids, acetylsalicylic acid, impaired kidney and liver functions, history of trauma, peritonitis, infection, pancreatitis, pelvic inflammatory disease, cancer, leukocytosis (>12,000/μL), and leukopenia (<3500/μL); (b) the presence of an additional cardiovascular risk factor such as hypertension, hyperlipidemia or coronary artery disease; (c) previously known diagnosis of diabetes mellitus; and (d) evidence of an underlying chronic inflammatory condition such as connective tissue disease and inflammatory bowel disease. The demographic, clinical, hematological, and biochemical data were extracted from the patients' charts. The NLR was calculated in patients and controls.

Data were presented as mean ± SD, median (min-max), or percentage. Correlations between the NLR and the various study parameters (except for history of cigarette smoking and intrauterine device use) were analyzed with Pearson or Spearman correlation tests as appropriate. Comparisons of study parameters of patients with ectopic pregnancy, miscarriage, or normal pregnancy were performed with a

Table 2. Selected hematological and biochemical data of patients with ectopic pregnancy, those with miscarriage, and those with normal pregnancy

	Normal pregnancy (n = 153)	Ectopic pregnancy (n = 152)	Miscarriage (n = 80)
White blood cells (10^3/mL)	8.27 ± 2.18	9.91 ± 3.66[a]	8.44 ± 2.05
Monocytes (10^3/mL)	0.47 ± 0.14	0.44 ± 0.16	0.46 ± 0.14
Neutrophils (10^3/mL)	5.65 ± 1.88	7.35 ± 3.60[b]	5.80 ± 1.81
Lymphocytes (10^3/mL)	2.11 ± 0.53	1.87 ± 0.71[c]	1.99 ± 0.61
Eosinophils (10^3/mL)	0.11 ± 0.09	0.10 ± 0.11	0.12 ± 0.10
Basophils (10^3/mL)	0.02 ± 0.01	0.02 ± 0.02	0.06 ± 0.40
Erythrocytes			
Red blood cells	4.5 ± 0.3	4.1 ± 0.6[d]	4.5 ± 0.9
Hemoglobin (g/dL)	13.0 ± 1.4[e]	11.6 ± 1.8	12.5 ± 1.5[f]
Hematocrit	38.6 ± 4.1	34.9 ± 5.4[g]	37.3 ± 3.8
Coagulation tests			
Platelet (10^3/μL)	273.8 ± 64.5	271.1 ± 75.2	272.7 ± 71.2
Prothrombin time	12.9 ± 1.3	12.8 ± 1.3	13.1 ± 1.5
APTT	30.2 ± 3.7	28.2 ± 3.9[h]	30.3 ± 4.1
INR	1.0 ± 0.1[i]	1.1 ± 0.1	1.1 ± 0.1
Biochemical tests			
FBG (mg/dL)	90.6 ± 18.2	110.6 ± 44.2[j]	97.3 ± 16.5
BUN (mg/dL)	8.9 ± 2.8	9.8 ± 3.3	9.5 ± 4.2
Creatinine (mg/dL)	0.59 ± 0.14	0.63 ± 0.13[k]	0.58 ± 0.13
ALT (U/L)	19.2 ± 14.3	16.6 ± 13.0	19.3 ± 18.3
AST (U/L)	19.1 ± 9.3	19.9± 13.1	20.9 ± 22.1

Data were presented as mean ± SD. [a,b,c,d,g,h,j,k]P<0.05 vs. miscarriage and normal pregnancy. [i,e]P<0.05 vs. miscarriage and ectopic pregnancy. [f]P<0.05 vs. ectopic pregnancy. INR, international normalized ratio; APTT, activated partial thromboplastin time; FBG, fasting blood glucose; BUN, blood urea nitrogen; ALT, alanine amino transferase; AST, aspartate aminotransferase.

one-way ANOVA with Tukey test, Kruskal-Wallis ANOVA with Mann-Whitney test, or chi-square test as appropriate. A *p* value of less than 0.05 was accepted as significant.

Results

In total, 385 pregnant women were included in our study: 153 with a healthy pregnancy, 152 with an ectopic pregnancy, and 80 with miscarriage. Fourteen women were excluded based on the aforementioned exclusion criteria or because of incomplete data collection. The clinical and biochemical characteristics of the study groups are summarized in **Tables 1** and **2**. The mean ages and BMIs of patients with normal pregnancy, those with ectopic pregnancy, and those with miscarriage were comparable (P>0.05). The mean gestational age of patients with miscarriage was significantly higher than that of patients with ectopic pregnancy and that of patients with normal pregnancy, and the mean gestational age of patients with ectopic pregnancy was also higher than that of patients with normal pregnancy (P<0.05). The mean gravidity of patients with ectopic pregnancy was significantly higher than that of patients with normal pregnancy and that of patients with miscarriage, and the mean gravidity of patients with miscarriage was also significantly higher than that of patients with normal pregnancy (P<0.05). The mean parity and history of cigarette smoking of patients with normal pregnancy were significantly lower than that of patients with ectopic pregnancy and that of patients with miscarriage (P<0.05); however, there was no significant difference between patients with ectopic pregnancy and patients with miscarriage with regard to the parity and history of cigarette smoking (P>0.05). There were no significant differences between the normal pregnancy, ectopic pregnancy, and miscarriage groups with regard to abortion and dilatation and curettage (P>0.05). The histories of ectopic pregnancy and of intrauterine device use of patients with ectopic pregnancy were significantly higher than that of patients with normal pregnancy and that of patients with miscarriage (P<0.05); however, there were no significant differences between patients with normal pregnancy and patients with miscarriage with regard to histories of ectopic pregnancy and of intrauterine device use (P>0.05).

The mean white blood cell count, neutrophil count, fasting blood glucose level, and creatinine content of patients with ectopic pregnancy was significantly higher than those of patients with normal pregnancy and those of patients with miscarriage (P<0.05). The mean lymphocyte, red blood cell and hematocrit count and

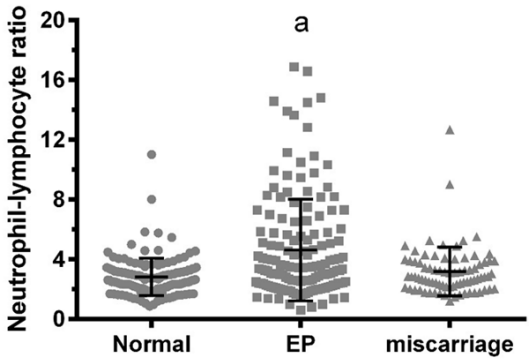

Figure 1. Neutrophil-to-lymphocyte ratios of patients with normal pregnancy (n = 153), those with ectopic pregnancy (n = 152), and those with miscarriage (n = 80). The horizontal lines and whiskers present the median and interquartile range, respectively. [a]P<0.05 vs. normal pregnancy and miscarriage.

activated partial thromboplastin time (APTT) of patients with ectopic pregnancy were significantly lower than those of patients with normal pregnancy and those of patients with miscarriage (P<0.05); however, there was no significant difference between patients with normal pregnancy and patients with miscarriage with regard to these variables (P>0.05). The mean hemoglobin level of patients with normal pregnancy was significantly higher than that of patients with ectopic pregnancy and that of patients with miscarriage, and the mean hemoglobin level of patients with miscarriage was also significantly higher than that of patients with ectopic pregnancy (P<0.05). The international normalized ratio (INR) of patients with normal pregnancy was significantly lower than that of patients with ectopic pregnancy and that of patients with miscarriage (P<0.05); however, there was no significant difference between patients with ectopic pregnancy and patients with miscarriage with regard to this variable (P>0.05). No significant differences were found between the study groups with regard to monocyte, eosinophil, basophil, and platelet counts; prothrombin time; and blood urea nitrogen, alanine amino transferase, and aspartate amino transferase levels (P>0.05).

Within each of the ectopic pregnancy, miscarriage, and normal pregnancy groups, we analyzed the correlations between the NLR and the clinical characteristics listed in **Tables 1** and **2**, and there were no meaningful relationships between the NLR and any of these variables (P>0.05).

Figure 1 presents the NLR of patients with ectopic pregnancy, miscarriage, and normal pregnancy. The mean NLR of patients with ectopic pregnancy was significantly higher than that of patients with normal pregnancy and that of patients with miscarriage (P<0.05). Although the mean NLR of patients with miscarriage was higher than that of patients with normal pregnancy, the difference was not statistically significant (P>0.05).

Figures 2 and **3** show the sensitivity and specificity of NLR of patients with ectopic pregnancy and of patients with miscarriage, respectively. For the ectopic pregnancy group, the sensitivity and specificity of the NLR with 3.0 as a cutoff value were 61%. For the miscarriage group, the sensitivity and specificity of the NLR with 2.8 as a cutoff value were 53%.

Discussion

In the current study, we assessed the diagnostic value of the NLR in patients with ectopic pregnancy and in patients with miscarriage. This is the first study to investigate the value of the NLR test in the differential diagnosis of ectopic pregnancy and miscarriage. Overall, although there were some differences between the study groups with regard to the demographic, clinical, hematological, and biochemical parameters, there were no meaningful effects of these parameters on the NLRs in the study groups. The mean NLR was higher in patients with ectopic pregnancy compared to that in patients with normal pregnancy; the mean NLR in patients with miscarriage was also higher than that of the normal pregnancy group, but not as high as the mean NLR in patients with ectopic pregnancy. Considering the sensitivity and specificity, overall, the NLR has a moderate diagnostic potential in patients with ectopic pregnancy and miscarriage, although it was lower in patients with miscarriage.

Lurie et al. [16] evaluated alterations in the leukocyte count and leukocyte differential in a large cohort of healthy women with an uncomplicated singleton pregnancy according to the trimester of pregnancy. They found that the leukocyte and neutrophil counts gradually and significantly increased from the first to the third trimester and that the lymphocyte count gradually decreased from the first to the second trimester. Overall, according to their study, in the first trimester, the neutrophil count was rela-

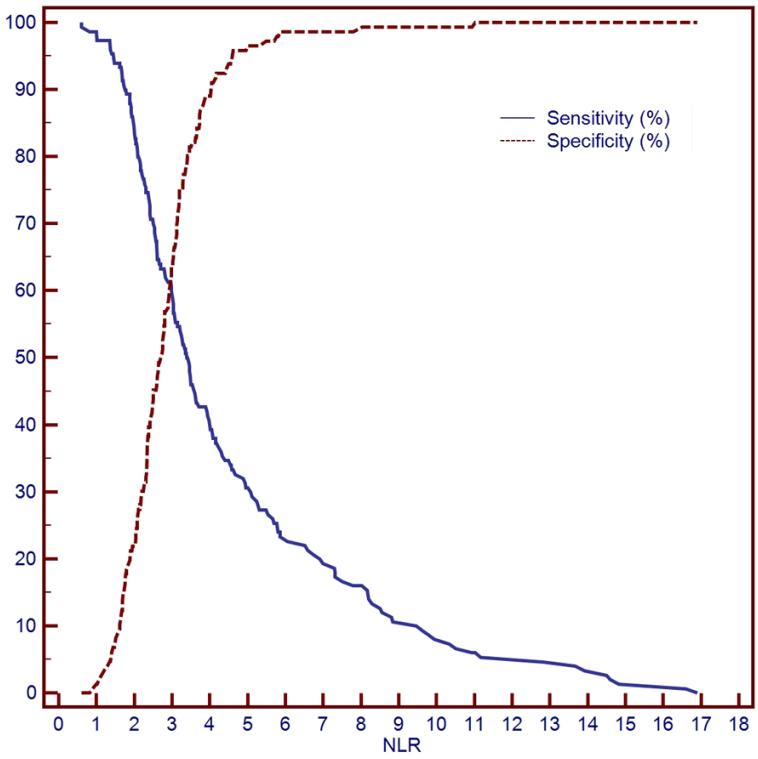

Figure 2. Sensitivity and specificity of neutrophil-lymphocyte ratio (NLR) of patients with ectopic pregnancy. The NLR with 3.0 as a cutoff value provided a sensitivity and a specificity of 61%.

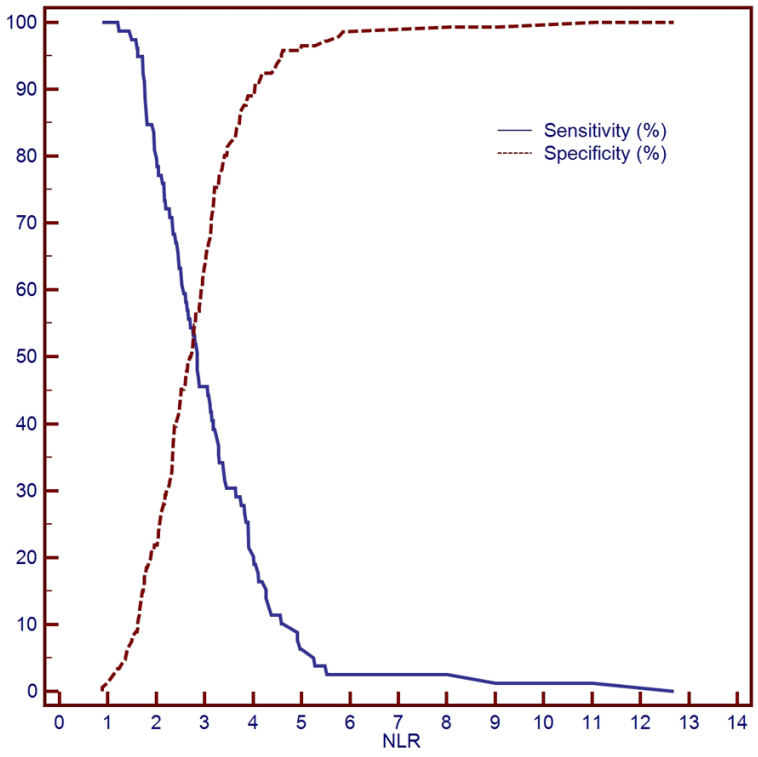

Figure 3. Sensitivity and specificity of neutrophil-lymphocyte ratio (NLR) of patients with miscarriage. The NLR with 2.8 as a cutoff value provided a sensitivity and a specificity of 53%.

tively low and the lymphocyte count was relatively high. Turgut et al. [17] compared changes in the mean platelet volume and leukocyte count in patients with ruptured tubal ectopic pregnancy with changes in those with unruptured tubal ectopic pregnancy. They found a statistically significant increase in the leukocyte count in patients with ruptured tubal ectopic pregnancy. Belo et al. [18] evaluated the serum C-reactive protein level and the neutrophil activation state during the three trimesters of normal pregnancies. They found that the leukocyte and neutrophil counts increased, while the lymphocyte count decreased, during both the first and second trimesters. The findings of Nasu et al. [19] suggest that the proportion of neutrophils increases but the proportion of lymphocytes decreases throughout pregnancy. As mentioned above, there are studies that investigated the differential leukocyte counts; however, there are no studies on the NLR in complicated or non-complicated pregnancies in the first trimester. Overall, in women with a first-trimester pregnancy, the NLR may change somewhat compared to that in non-pregnant women, but we think that this difference does not detract from the diagnostic value of the NLR in the differential diagnosis of ectopic pregnancy and miscarriage.

There are some limitations to this study. Cause and effect could not be established because this study was a retrospective analysis. Some limitations relate to the nature of ectopic pregnancy, which can be intact or not intact because of a tubal abortion or a recent

(within a few weeks) rupture. It is not easy to make a differential diagnosis on the basis of clinical presentation. As regards limitations related to miscarriage, patients can present with vaginal bleeding a few weeks after the death of the embryo or fetus. In both patients with ectopic pregnancy and those with miscarriage, it may be difficult to determine the actual gestational age.

An important goal in the evaluation of women with bleeding in early pregnancy is to exclude the possibility of ectopic pregnancy, because ruptured ectopic pregnancy can result in severe hemorrhage and death. Although the power of the NLR for detecting active ectopic pregnancy is suboptimal according to our findings, the NLR could be an important measure of systemic inflammation, as it is cost-effective, readily available as a noninvasive marker, and easily calculated. We found that the NLR is not useful as a diagnostic marker for miscarriage. Further work is needed to determine the clinical applicability of the NLR as a diagnostic tool to differentiate between ectopic pregnancy and miscarriage in women with vaginal bleeding.

Conclusions

Ectopic pregnancy is more common in women with a markedly increased NLR. The NLR with a standardized threshold level has potential as a valuable part of the diagnostic workup to assess the risk of ectopic pregnancy in women without infection, though confirmation from further studies is needed. Miscarriage may not result in an increase in the NLR.

Disclosure of conflict of interest

None.

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