European Journal of Obstetrics & Gynecology and Reproductive Biology 180 (2014) 12–15

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Neutrophil-to-lymphocyte ratio as a diagnostic marker of intrahepatic cholestasis of pregnancy

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ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 23 May 2014
Accepted 28 May 2014

Keywords:
Inflammation
Aminotransferase
Hepatocellular injury
Cholestasis of pregnancy

ABSTRACT

Introduction: In this study, we aimed to investigate the relationship between neutrophil-to-lymphocyte ratio (NLR) and total bile acid (TBA) concentration in pregnant women with intrahepatic cholestasis of pregnancy (ICP).
Materials and methods: Fasting and postprandial TBA, NLR, and aminotransferase (AST/ALT) levels in the blood samples of 65 pregnant women with intrahepatic cholestasis were examined in this prospective case–control study. Thirty-three of the patients had mild disease and 32 had severe disease; 70 healthy women in uncomplicated pregnancies served as the control group.
Results: Not only was the mean NLR elevated in the pregnant women with cholestasis when compared to the controls, but it also predicted the severity of the cholestasis. The correlation between fasting TBA and NLR was significant.
Comments: Although TBA is still the diagnostic standard, NLR can be used as an initial screening tool due to its high specificity.

Background and objectives

Intrahepatic cholestasis of pregnancy (ICP) is a medical condition characterized by elevated liver enzyme and serum bile acid levels, as well as intense itching localized to the abdomen, legs, palms, and soles [1–3]. ICP can lead to complications for both mother and fetus, such as fetal distress, perinatal mortality, stillbirth, and preterm labor [4–6].

The etiology of ICP is still not fully understood. Genetic, endocrinologic, nutritional, and environmental factors are likely to be important in the pathogenesis of the disease. The association between inflammation and cholestatic liver disease has been previously described [7–9]. It has been reported that elevated bile acid levels trigger an inflammatory response, causing hepatocellular injury. Systemic inflammation can be measured by using a variety of biochemical and hematological markers. Although novel disease-specific biomarkers have been identified, most of them, including the measurement of total bile acid (TBA) concentration, are time consuming and expensive. Recent evidence indicates that measuring the ratio of blood cell subtypes, such as the neutrophil-to-lymphocyte ratio (NLR), might have prognostic significance for diseases related to chronic low-grade inflammation [10,11]. In addition, because it is readily available and easily calculated, this method might be a promising alternative diagnostic tool for diseases associated with chronic low-grade inflammation, such as ICP [10,11]. However, little is known and has been published about NLR and its relationship with ICP; therefore, the present study was conducted to evaluate the efficacy of NLR for the diagnosis and management of ICP.

Materials and methods

This prospective case–control study was conducted at the perinatology department of Dr. Zekai Tahir Burak Women's Health Care, Education and Research Hospital, Ankara, Turkey. The study was approved by the Institutional Review Board of Dr. Zekai Tahir Burak Women's Hospital.

Sixty-five pregnant women with ICP, 33 with mild disease and 32 with severe disease, were recruited for the study between June 2013 and January 2014.

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http://dx.doi.org/10.1016/j.ejogrb.2014.05.042
0301-2115/

The control group consisted of 70 healthy women uncomplicated pregnancies in third trimester, matched for maternal ages, during the same period.

Inclusion criteria for the study group included itching without rash in different parts of the body, severe enough to require hospital admission, and elevated total bile acid (TBA) ($\geq$10 μmol/L) and/or aminotransferase levels in the blood sample.

All the pregnants with ICP were hospitalized and tested for hepatitis A, B and C virus as part of initial evaluation. Routine urine cultures were obtained and daily body temperature were measured for all the patients. However, further investigations of infection markers are made on clinical ground. Patient with signs and symptoms of active infection are excluded from the study. In addition, patients with multiple gestation, with chronic inflammatory diseases like ulcerative colitis, Crohn's diseases, rheumatoid arthritis, vasculitis, familial Mediterranean fever or a history of systemic disease other than ICP, or in active labor were excluded from the study.

Blood samples were obtained from the antecubital vein early in the morning, following ten hours of fasting, and two-hour postprandial and TBA levels were determined by enzymatic assay. The patients were classified as having mild or severe cholestasis based on TBA concentrations of 10–40 or $\geq$40 μmol/L, respectively [1,2]. NLR was calculated as the ratio of absolute neutrophil count to absolute lymphocyte count, both obtained from the same blood sample. All the other blood analyses were carried out within two hours of blood sampling using a Beckman-Coulter Gen-S system device at the central laboratories of Dr. Zekai Tahir Burak Hospital, Ankara. The following clinical and demographic data were obtained: maternal age, obstetric history, and presence of maternal and neonatal complications.

The statistical analyses were conducted using the Statistical Package for the Social Sciences version 12.0 for Windows (SPSS, Chicago, IL). The data were summarized as mean ± standard deviation and median (minimum–maximum). Proportions were compared with Fisher's exact test or the chi-square test where appropriate. For parametric variables, one-way ANOVA was used for comparing three independent groups, and post hoc comparisons were performed with Tukey's HSD test. The Mann–Whitney *U* test was used for nonparametric variables in two independent groups. Spearman's rank correlation was used to assess the relationship between quantitative variables.

The dependence of fasting and postprandial serum TBA levels and NLR was evaluated by linear regression analysis. Receiver operating characteristic (ROC) analysis was used to evaluate the performance of NLR in the diagnosis of ICP and in the detection of severe ICP based on fasting serum TBA concentrations. Statistical significance was set at $p \leq 0.05$.

Results

The demographic and obstetric data of the ICP and control groups are shown in Table 1. While mean white blood cell count was significantly higher in the group of women with severe cholestasis compared to the control group, their lymphocyte count was significantly lower. The mean gestational age, neutrophil counts, neutrophil lymphocyte ratios, *alanine* aminotransferase, and aspartate aminotransferase levels were significantly different within each group. The mean NLR was significantly different in each group. NLR levels were higher in the mild ICP group than in the control group and higher in the severe ICP group than in both of the other groups (Table 1).

Serum TBA concentrations in the mild and severe ICP groups are presented in Table 2.

The ROC analysis of the diagnostic performance of NLR for ICP is shown in Fig. 1. The area under the curve was 0.92 ± 0.03 (95% confidence interval (CI) 0.861–0.969). The best NLR cutoff value was 2.93. The ratio above this value had 91% sensitivity, 84% specificity, 83% positive predictive value, and 91% negative predictive value for the diagnosis of ICP.

The ROC curve analysis was also performed to assess the predictive value of NLR in differentiating minimal and severe cholestasis. The area under the curve was 0.75 ± 0.060 (95% CI 0.638–0.872) (Fig. 2). The best NLR cutoff value in predicting the severity of ICP was 4.05, above which the sensitivity and specificity were 78% and 67% and the positive and negative predictive values were 69% and 76%, respectively.

Multivariable logistic regression was used to verify the association between serum TBA levels and NLR further, and it demonstrated a positive correlation between the two. Regression equality was obtained at NLR = 3.352 + (0.034 × fasting bile acid) (Fig. 3). Only the correlation between fasting TBA (not postprandial TBA) and NLR was significant (regression coefficient 0.034; $p < 0.001$).

Comments

Because ICP is also an inflammatory process, in the search of a cost-effective, readily available laboratory test, we decided to conduct the current study, using NLR to investigate its role in the diagnosis of ICP. We aimed to study the possible relationship

Table 1
Demographic, obstetric, and laboratory characteristics of intrahepatic cholestasis (ICP) and control study populations (mean ± SD).

Clinical and laboratory parameters	Normal pregnancy $n = 70$	Mild cholestasis $n = 33$	Severe cholestasis $n = 32$	*P* value
Age (years)	28.1 ± 4.7	28.8 ± 4.8	28.9 ± 5.3	0.686
Gestational week	38.7 ± 1.1[b,c]	37.2 ± 1.7[a,c]	35.6 ± 2.7[a,b]	<0.001
BMI	28.9 ± 3.8	28.5 ± 3.9	29.6 ± 4.2	0.915
Gravidity	1.6 ± 0.7	1.8 ± 0.9	1.6 ± 0.7	0.530
Parity	0.6 ± 0.6	0.7 ± 0.8	0.5 ± 0.6	0.465
AST (U/L)	22.4 ± 7.1[c]	44.8 ± 38.3[c]	176.0 ± 94.7[a,b]	<0.001
ALT (U/L)	23.0 ± 9.5[c]	61.4 ± 59.4[c]	225.7 ± 166.7[a,b]	<0.001
WBC × 10^9/L	8.32 ± 1485	8.63 ± 1696.8	9.12 ± 1403.5	0.44
Neutrophils × 10^9/L	5.2 ± 1.6[b,c]	6.7 ± 2[a,c]	7.991 ± 2[a,b]	<0.001
Lymphocytes × 10^9/L	2.4 ± 0.8[c]	2 ± 1.4	1.6 ± 0.4[a]	<0.001
NLR	2.32 ± 0.77[b,c]	3.97 ± 1.5[a,c]	5.59 ± 1.98[a,b]	<0.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; NLR, neutrophil/lymphocyte ratio; ICP, intrahepatic cholestasis of pregnancy; WBC, white blood cell.

[a] Different from the control group.
[b] Different from the mild ICP group.
[c] Different from the severe ICP group.