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Correlation between hematological parameters and ancylostomiasis: A retrospective study

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Objective: Our aim intended to determine the relationship between hematological parameters (neutrophil-to-lymphocyte ratio [NLR], platelet-to-lymphocyte ratio [PLR], and eosinophil-to-lymphocyte ratio [ELR]) and ancylostomiasis.

Methods: There were 140 patients with ancylostomiasis and 159 healthy controls enrolled in this study. All data were collected from electronic medical records of the First Affiliated Hospital of Guangxi Medical University.

Results: The levels of NLR, PLR, and ELR in ancylostomiasis patients were significantly higher than those in the healthy controls (all $P = 0.000$). A receiver operating characteristic curve was generated to assess the diagnostic efficacy of these three hematological parameters. ELR (AUC = 0.850; sensitivity = 75.00%; specificity = 86.80%) showed the superior AUC than those of NLR (AUC = 0.718; sensitivity = 53.57%; specificity = 88.68%) and PLR (AUC = 0.806; sensitivity = 68.57%; specificity = 86.79%), respectively. A multivariate regression model using the two selected indices (RBC and ELR) was established with the model's sensitivity and specificity reached 82.86% and 96.23%, respectively. In the ancylostomiasis patient group, NLR ($r = -0.452$, $P = 0.000$) and PLR ($r = -0.357$, $P = 0.000$) were reversely associated with eosinophils.

Conclusion: The pretreatment levels of the three hematological parameters (NLR, PLR, and ELR) may serve as valuable indicators for distinguishing patients with ancylostomiasis from healthy controls. NLR and PLR are negatively associated with the previous indicator, eosinophils.

KEYWORDS
ancylostomiasis, eosinophil-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

1 | INTRODUCTION

Ancylostome is one of the most prevalent soil-borne nematodes in rural areas in China, especially in tropical and subtropical areas with scarce resources and poor sanitary conditions.[1-3] Among the parasitic nematodes in the digestive tract of the human body, ancylostomes are the most harmful, causing severe chronic blood loss and multisystem diseases.[4,5] Ancylostomiasis is one of the five major parasitic diseases in China, where it is more common in environments with a warm climate, abundant rainfall, and fertile soil.[6-9]

Inflammatory response plays an indispensable role in the pathogenesis and severity of ancylostomiasis.[10] The correlation between a systemic inflammation marker such as C-reactive protein and ancylostomiasis has been reported.[11] More recently, some hematological parameters related to inflammation have received extensive attention, which include the neutrophil-to-lymphocyte ratio (NLR), the

TABLE 1 Characteristics of patients with ancylostomiasis

Characteristics	Median range or number (%) (n: 140)
Gender	
Male	51 (36.43%)
Female	89 (63.57%)
Age (y)	59.00 (1.00-85.00)
Clinical symptoms	
Anemia	90 (64.28%)
Mild anemia	47 (33.57%)
Moderate anemia	35 (25.00%)
Severe anemia	8 (5.71%)
Fecal occult blood test	
Positive	20 (14.28%)
Negative	120 (85.71%)
Hypoproteinemia	104 (74.28%)
Abdominal discomfort	94 (67.14%)
Weight loss	81 (57.86%)
Hypodynamia	61 (43.57%)
Cough	20 (14.28%)
Dermatitis	14 (10.00%)

platelet-to-lymphocyte ratio (PLR), and the eosinophil-to-lymphocyte ratio (ELR). The predictions and diagnostic significance of these three parameters have been evaluated in a variety of diseases, such as metastatic melanoma, systemic lupus erythematosus (SLE), and Clonorchis sinensis infection.[12-14] Nevertheless, the clinical value of these three hematological parameters (NLR, PLR, and ELR) in ancylostomiasis is still unclear.

Hence, our study intended to investigate the association between these three hematological parameters (NLR, PLR, and ELR) and ancylostomiasis. We established a model with superior AUC, sensitivity, and specificity for differentiating ancylostomiasis patients from healthy controls.

2 | MATERIALS AND METHODS

2.1 | Patients

There were 140 patients with ancylostomiasis and 159 healthy controls included in this study. All the data were collected from the electronic medical records of the First Affiliated Hospital of Guangxi Medical University from 2013 to 2017. The patient group was diagnosed by microscopic examination of ancylostome ova. Patients were excluded if they met any of the following criteria: (a) with other parasitic infection or co-infection; (b) HBV, HIV, syphilis, or other viral infections; (c) hypertension or diabetes; (d) neoplastic diseases; (e) potential immune-related diseases; (f) autoimmune diseases; or (g) blood system diseases such as leukemia; or (h) kidney diseases. The control group was matched with the patient group in gender and age. Additionally, all healthy controls were selected from the Physical Examination Center of our hospital. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, and the informed consent of all participants was obtained.

2.2 | Data collection

The following data from healthy controls and patients before treatment were collected: gender, age, white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils (NEU), lymphocytes (LYM), and eosinophils (EOS).

2.3 | Laboratory measurements

Routine blood parameters were measured using the Beckman Coulter LH 780 blood analyzer (Beckman Coulter, Brea, CA, USA). NLR (neutrophils/lymphocytes), PLR (platelets/lymphocytes), and ELR (eosinophils/lymphocytes) were calculated based on the blood cell counts accordingly. The colloidal gold method was used to detect fecal occult blood.

2.4 | Statistical analysis

Kolmogorov-Smirnov test was used to evaluate the distribution status of the collected data. The normal distribution data were expressed by mean and standard deviation (mean ± SD), and the non-normal distribution data were expressed by the median and interquartile range (IQR). The clinical characteristics of the ancylostomiasis patients were described by quantity and proportion. To compare the statistical differences between the two groups, a Student *t* test and Mann-Whitney *U* test were used to compare the quantitative data as appropriate, while a chi-square test was used to compare the categorical variables. Univariate and multivariate analysis served to determine the independent risk factors for ancylostomiasis. Spearman's correlation coefficient was performed for inspecting the relativity between two variables. A model derived from multivariate logic regression. A receiver operating characteristic (ROC) curve and the area under the ROC curve (AUROC) were generated to assess the diagnostic efficiency of NLR, PLR, ELR, and the multivariate regression model. The statistical analysis and graphical processing of all data were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA), GraphPad Prism 5, and MedCalc statistical software (version 11.3.8.0). The value of $P < 0.05$ (two-tailed) was considered to be statistically significant.

3 | RESULTS

3.1 | Clinical characteristics of ancylostomiasis patients

The clinical characteristics of the ancylostomiasis patients were summarized in Table 1. There were 140 patients included in this study

TABLE 2 Comparison of laboratory parameters in ancylostomiasis patients with those of healthy controls

	Patients (n = 140)	Controls (n = 159)	*P*-value
Gender(male/female)	51/89	61/98	0.730
Age (y)	59.00 (50.00, 65.00)	62.00 (42.00, 67.00)	0.139
WBC ($\times 10^9$/L)	6.39 (4.88, 8.96)	6.29 (5.61, 7.28)	0.937
RBC ($\times 10^{12}$/L)	3.87 ± 0.80	4.53 ± 0.29	0.000
HGB (g/L)	103.44 ± 24.96	135.48 ± 8.31	0.000
MCV (fL)	86.88 (73.42, 91.78)	90.04 (87.51, 92.22)	0.000
MCH (pg)	28.36 (22.96, 30.40)	29.91 (29.04, 30.82)	0.000
MCHC (g/L)	325.05 (312.50, 332.15)	332.80 (329.00, 335.80)	0.000
PLT ($\times 10^9$/L)	255.30 (209.62, 320.10)	232.30 (206.70, 260.90)	0.002
NEU ($\times 10^9$/L)	3.64 (2.59, 5.33)	3.58 (2.93, 4.13)	0.327
LYM ($\times 10^9$/L)	1.36 (1.06, 1.87)	2.15 (1.86, 2.40)	0.000
EOS ($\times 10^9$/L)	0.35 (0.15, 0.72)	0.11 (0.07, 0.20)	0.000
NLR	2.60 (1.63, 4.47)	1.61 (1.36, 2.06)	0.000
PLR	171.52 (129.74, 269.48)	106.83 (90.00, 131.76)	0.000
ELR	0.26 (0.13, 0.48)	0.06 (0.03, 0.09)	0.000

ELR, eosinophil-to-lymphocyte ratio; EOS, eosinophils; HGB, hemoglobin; LYM, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NEU, neutrophils; NLR, neutrophil-to-lymphocyte ratio; Patients, ancylostomiasis patients; PLR, platelet-to-lymphocyte ratio; PLT, platelet count; RBC, red blood cells; WBC, white blood cells.

with average age of 59 years. Male and female accounted for 36.43% and 63.57%, respectively. Of those, 90 patients (64.28%) had anemia, while only 5.71% of the ancylostomiasis patients showed severe anemia. The positive rate of the fecal occult blood test was only 14.28%. Hypoproteinemia was seen in 74.28% of the ancylostomiasis patients. Ninety-four (67.14%) patients had clinical manifestations of abdominal discomfort, 81 (57.86%) had weight loss, 61 (43.57%) had hypodynamia, 20 (14.28%) had cough, and 14 (10.00%) had dermatitis.

3.2 | Comparison of hematological parameters between ancylostomiasis patients and healthy controls

As shown in Table 2, WBC ($P = 0.937$) and neutrophils ($P = 0.327$) were not statistically different between ancylostomiasis patients and the healthy controls. However, for the patient group, the median (25th-75th IQR) MCV, MCH, and MCHC were 86.88 (73.42, 91.78) fL, 28.36 (22.96, 30.40) pg, and 325.05 (312.50, 332.15) g/L, respectively. For the control group, the median (25th-75th IQR) MCV, MCH, and MCHC were 90.04 (87.51, 92.22) fL, 29.91 (29.04, 30.82) pg, and 332.80 (329.00, 335.80) g/L, respectively. Therefore, the ancylostomiasis patients showed lower MCV, MCH, and MCHC levels than those observed in healthy controls ($P = 0.000$). The RBC and HGB values of ancylostomiasis patients were also significantly lower than those of the controls (RBC $3.87 \pm 0.80 \times 10^{12}$/L vs $4.53 \pm 0.29 \times 10^{12}$/L, $P = 0.000$; HGB 103.44 ± 24.96 g/L vs 135.48 ± 8.31 g/L, $P = 0.000$, respectively). The PLT, NEU, and EOS counts of ancylostomiasis patients were also considerably higher than those for the healthy controls (PLT 255.30 [209.62, 320.10] $\times 10^9$/L vs 232.30 [206.70, 260.90] $\times 10^9$/L, $P = 0.002$; NEU 3.64 [2.59, 5.33] $\times 10^9$/L vs 3.58 [2.93, 4.13] $\times 10^9$/L, $P = 0.000$; EOS 0.35 [0.15, 0.72] $\times 10^9$/L vs 0.11 [0.07, 0.20] $\times 10^9$/L, $P = 0.000$, respectively), while the LYM counts in the patient group showed a low level compared to the control group (LYM 1.36 [1.06, 1.87] $\times 10^9$/L vs 2.15 [1.86, 2.40] $\times 10^9$/L, $P = 0.000$).

3.3 | Comparison of candidate markers (NLR, PLR, and ELR) between ancylostomiasis patients and healthy controls

In the present research, we evaluated the differences in three candidate markers (NLR, PLR, and ELR) between patients and controls. As shown in Figure 1, the levels of the three candidate markers in the disease group increased significantly compared with those of the control group ($P = 0.000$); in particular, the ancylostomiasis patients had substantially increased ELR levels in comparison with the healthy controls ($P = 0.000$). The above results indicate that NLR, PLR, and ELR have important significance for the identification of ancylostomiasis.

3.4 | Establishment of a potential multivariate logistic regression model for distinguishing ancylostomiasis patients from healthy controls

The relationship between ancylostomiasis and hematological parameters was determined by univariate analysis, and then,

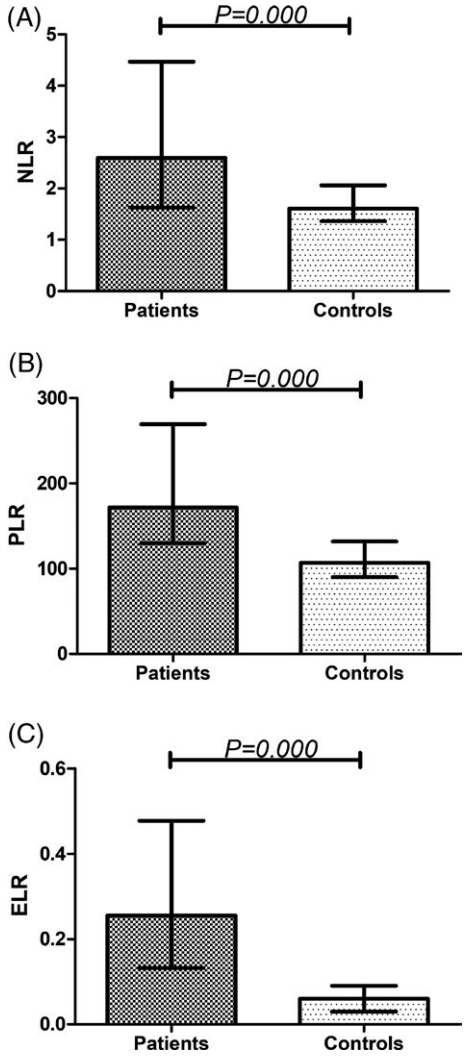

FIGURE 1 Comparison of NLR (A), PLR (B), ELR (C) in ancylostomiasis patients and healthy controls. ELR, eosinophil-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio

statistically significant variables were selected for multivariate analysis. The results of the univariate analysis are presented in Table 3, which reveals that RBC (OR = 0.108, 95% CI = 0.059-0.197, $P = 0.000$), MCV (OR = 0.899, 95% CI = 0.867-0.933, $P = 0.000$), MCH (OR = 0.719, 95% CI = 0.647-0.800, $P = 0.000$), MCHC (OR = 0.898, 95% CI = 0.869-0.928, $P = 0.000$), NLR (OR = 2.372, 95% CI = 1.769-3.180, $P = 0.000$), PLR (OR = 1.025, 95% CI = 1.018-1.031, $P = 0.000$), and ELR (OR = 1.134×10^7, 95% CI = 0.015×10^7-8.510×10^8, $P = 0.000$) can be used as potential independent predictors of ancylostomiasis epidemics. Then, the seven significant variables were analyzed by multivariate analysis. The results showed that RBC ($\beta = -3.094$, $P = 0.000$) and ELR ($\beta = 20.722$, $P = 0.000$) were significantly correlated with the occurrence of ancylostomiasis; thus, we obtained the optimal model (logit $P = 20.722 \times \text{ELR} - 3.094 \times \text{RBC} + 10.431$) for differentiating ancylostomiasis patients from healthy controls.

3.5 | Establishment of the AUC, sensitivity, and specificity of EOS, NLR, PLR, ELR, and logit *P* for the identification of ancylostomiasis

The ROC curve was created by comparing the hematological parameters of patients with ancylostomiasis with those of healthy controls (Figure 2). The maximum Youden index obtained by the ROC curves determined the optimal cutoff values of five candidate markers. Our results demonstrated that the cutoff values of EOS, NLR, PLR, ELR, and the multivariate regression model were 0.29, 2.38, 145.99, 0.13, and 0.12, respectively. For EOS, the area under the ROC curve (AUC) value was 0.761 (95% CI 0.708-0.808; sensitivity = 57.14%; specificity = 89.31%). For NLR, the AUC value was 0.718 (95% CI 0.663-0.768; sensitivity = 53.57%; specificity = 88.68%). The AUC value of PLR was 0.806 (95% CI 0.756-0.849; sensitivity = 68.57%; specificity = 86.79%). ELR produced an AUC value of 0.850 (95% CI 0.804-0.888; sensitivity = 75.00%; specificity = 86.80%). In particular, the multivariate logistic regression model (logit *P*) indicated the best distinction between ancylostomiasis patients and healthy controls, and its AUC value was 0.939 (95% CI 0.906-0.963; sensitivity = 82.86%; specificity = 96.23%).

3.6 | Correlations of NLR and PLR with EOS in patients with ancylostomiasis

The association of EOS with NLR and PLR in the patient group was presented in Figure 3. There was a significant negative correlation between NLR and EOS ($r = -0.452$, $P = 0.000$, Figure 3A). A significant negative association was also observed between PLR and EOS in ancylostomiasis patients ($r = -0.357$, $P = 0.000$, Figure 3B).

4 | DISCUSSION

Ancylostomiasis is characterized by long-term chronic blood loss and the initiation of multiple systemic diseases (such as diseases of the respiratory, digestive, and cardiovascular systems).[4,5,15] A definitive diagnosis of ancylostomiasis and early treatment are critical in control the development of the disease and avoiding complications affecting multiple systems, thus further reducing the financial burden of patients.

Inflammation plays a major role in the pathogenesis of ancylostome.[10] For our research, the diagnostic efficacy of NLR, PLR, and ELR in ancylostomiasis was investigated. Our findings showed that the NLR, PLR, and ELR values of patients were significantly higher than those of controls. This suggests that these three inflammatory markers (NLR, PLR, and ELR) are statistically significant in differentiating patients with ancylostomiasis from healthy controls.

The mechanisms of the hematological change of these parameters and ancylostomiasis were unclear. Studies by Veraldi et al showed that neutrophils and lymphocytes were involved in the development of the ancylostome infection and that these cells were found in some tissue fluids.[16-18] Wiwanitkit et al[19] revealed that platelets were

TABLE 3 Univariate and multivariate analyses used for differentiating significant predictors to distinguish ancylostomiasis patients from healthy controls

	Univariate analysis			Multivariate analysis		
Variables	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Gender	1.086	0.679-1.738	0.730			
Age (y)	1.009	0.992-1.025	0.139			
RBC ($\times 10^{12}$/L)	0.108	0.059-0.197	0.000	0.047	0.013-0.175	0.000
MCV (fL)	0.899	0.867-0.933	0.000	0.016	0.000-6.471	0.177
MCH (pg)	0.719	0.647-0.800	0.000	1.790×10^{5}	$0.003\text{-}1.261 \times 10^{13}$	0.190
MCHC (g/L)	0.898	0.869-0.928	0.000	0.304	0.059-1.564	0.154
NLR	2.372	1.769-3.180	0.000	2.071	0.989-4.333	0.053
PLR	1.025	1.018-1.031	0.000	1.015	1.000-1.030	0.053
ELR	1.134×10^{7}	$0.015 \times 10^{7}\text{-}8.510 \times 10^{8}$	0.000	4.746×10^{9}	$1.584 \times 10^{6}\text{-}1.422 \times 10^{13}$	0.000

ELR, eosinophil-to-lymphocyte ratio; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RBC, red blood cells.

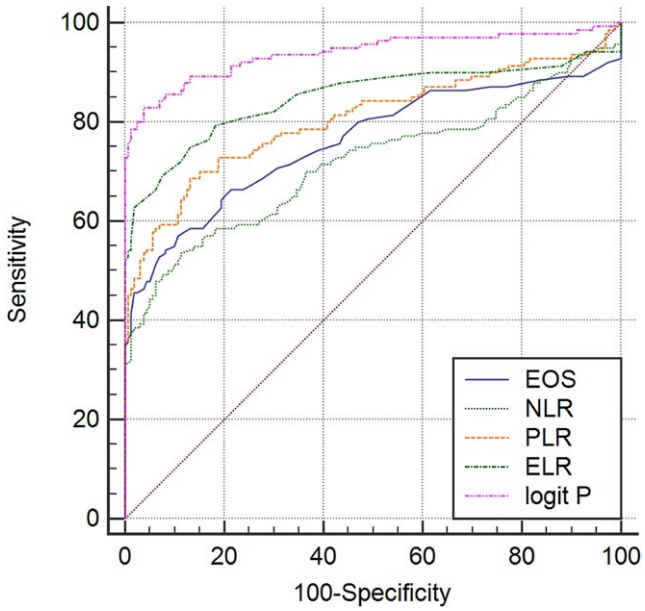

FIGURE 2 Receiver operating characteristic curves of EOS, NLR, PLR, ELR, and the logit *P* for distinguishing ancylostomiasis patients from healthy controls. ELR, eosinophil-to-lymphocyte ratio; EOS, eosinophils; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio

associated with ancylostomiasis. We observed elevated neutrophils and platelets and decreased lymphocytes in the patient group in comparison with the healthy controls. Our findings are consistent with previous studies discussed above. After the organism was infected with the ancylostome, the chemotactic neutrophils concentrated heavily on the local lesion and carried out active phagocytosis and secretion. Platelets were concentrated in hemostasis, and reduced lymphocytes caused a decrease in body immune function. This may explain the clinical value of elevated NLR and PLR for ancylostomiasis.

Eosinophils were closely related to ancylostome infection.[20,21] And from the standard interpretation of the diagnosis of ancylostomiasis (WS 439-2013), we found that eosinophils were included in the diagnostic criteria for ancylostomiasis. In our research results, NLR, PLR, and EOS were significantly correlated in patients group; therefore, the clinical values of the two inflammatory markers (NLR and PLR) were investigated. More importantly, PLR performed with good identification of ancylostomiasis, which was comparable to or even beyond that of EOS.

Microscopic examination of parasite eggs has been the gold standard for the diagnosis of most parasitic diseases, and ancylostomiasis has been no exception.[22,23] However, a study by Walana et al[24] showed that the positive rate for parasite eggs was only 0.3%; this positive rate is hyperbolically low compared to 47 147 patients with intestinal parasite infection. Thus, the miss rate for microscopic examination of parasitic eggs is extremely high. There are also some inadequacies in the microscopic examination of parasitic eggs; this examination method requires some dangerous chemicals, such as concentrated hydrochloric acid and ether, which will cause harm to the human body. Also, a large amount of time is required to complete the examination, which affects the progress of inspectors and doctors in diagnosing the disease. In contrast, blood routine parameters such as ELR and PLR are relatively easy to obtain and fast to detect, and they have a wide range of clinical applications. Hence, ELR and PLR may be alternative or complementary choices.

The diagnostic value of ELR has been rarely studied.[14] Our study demonstrates similar findings as shown by others the diagnostic and recurrence prediction value of ELR in nasal diseases, such as nasal polyposis and sinonasal polyps.[25,26] Moreover, ELR (AUC = 0.850; sensitivity = 75.00%; specificity = 86.80%) performed with the most superior AUC compared with NLR (AUC = 0.718; sensitivity = 53.57%; specificity = 88.68%) and PLR (AUC = 0.806; sensitivity = 68.57%; specificity = 86.79%). The clinical significance and application of ELR in different diseases will be an interesting topic to study in the future. After univariate and multivariate analysis, we also found that increased RBC and ELR are risk factors for ancylostomiasis. Based on these two

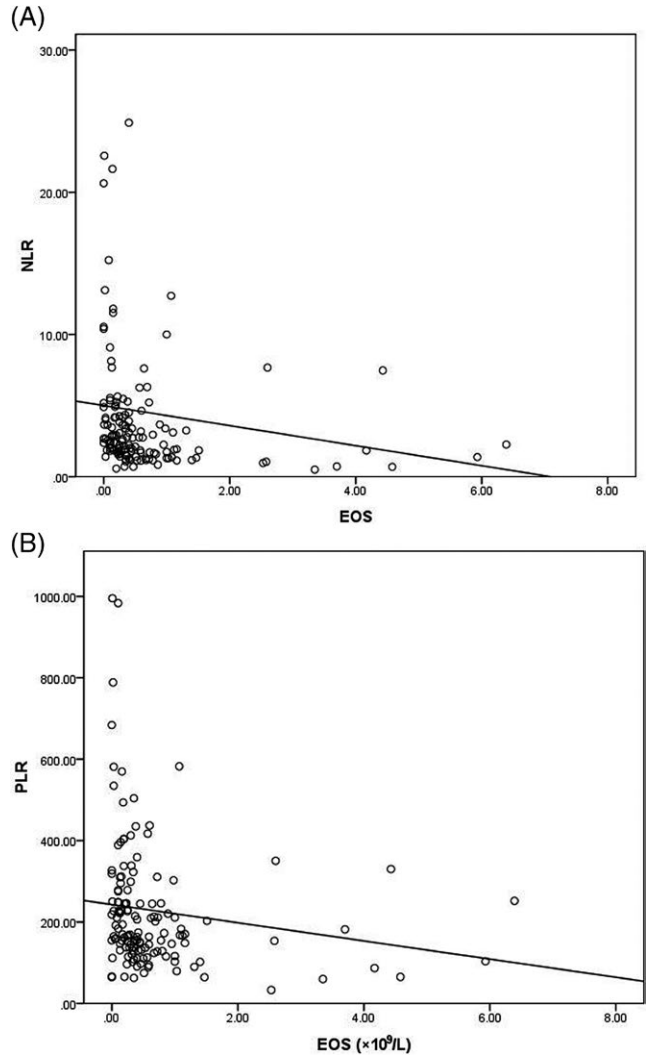

FIGURE 3 Correlation analysis between NLR and EOS, PLR and EOS in patients with ancylostomiasis. A, NLR and EOS in patients with ancylostomiasis; B, PLR and EOS in patients with ancylostomiasis. ELR, eosinophil-to-lymphocyte ratio; EOS, eosinophils; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio

indicators, we established a multivariate regression model: logit $P = 20.722 \times ELR - 3.094 \times RBC + 10.431$. The value of this model (AUC = 0.939; sensitivity = 82.86%; specificity = 96.23%) in differentiating ancylostomiasis patients from healthy controls was superior to that of NLR, PLR, and ELR. Our findings demonstrate a number of advantages: Firstly, we first proposed the use of routine blood parameters (NLR, PLR, and ELR) to identify ancylostomiasis. Secondly, for the first time, our research has established a model with excellent clinical performance for distinguishing ancylostomiasis patients from healthy controls. Finally, we are the first to study the relationship between NLR, PLR, and EOS.

Nevertheless, there are also limitations to our study. First of all, our research is a retrospective analysis, so it has some unavoidable inherent defects, such as recall bias and selection bias. Second, the sample size is relatively small with only 140 patients, due to the low prevalence of ancylostomiasis in our region. Thirdly, our research is a single-center study, which does not reflect the overall situation of ancylostomiasis. Consequently, our findings need to be validated further by multicenter and large population in prospective studies.

In conclusion, our results reveal that the pretreatment values of NLR, PLR, and ELR may be helpful in the identification of ancylostomiasis. NLR and PLR have a negative correlation with the EOS. ELR may be an independent risk factor for ancylostomiasis and serve as the most effective indicator to distinguish patients with ancylostomiasis from healthy controls.

AUTHORS' CONTRIBUTIONS

XQ and SL drafted the overall design of this paper as the co-corresponding authors. ZH and HC wrote the article. LH, SC, and ZH collected the laboratory data. SQ and JZ analyzed the data.

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