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The Relationship Between ^{14}C Urea Breath Test Results and Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios

^{14}C Üre Nefes Testi Sonuçları ile Nötrofil/Lenfosit ve Trombosit/Lenfosit Oranları Arasındaki İlişki

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Abstract

Aim: Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) are used as inflammatory markers in several diseases. However, there are little data regarding the diagnostic ability of NLR and PLR in *Helicobacter pylori*. We aimed to assess the association between the ^{14}C urea breath test (^{14}C-UBT) results and NLR and PLR in *H. pylori* diagnosis.

Methods: Results of 89 patients were retrospectively analysed in this study. According to the ^{14}C-UBT results, patients were divided into two groups: *H. pylori* (+) and *H. pylori* (-) (control group). Haematological parameters, including hemoglobine, white blood cell (WBC) count, neutrophil count, lymphocyte count, NLR, platelet count, and PLR were compared between the two groups.

Results: The mean total WBC count, neutrophil count, NLR and PLR in *H. pylori* (+) patients were significantly higher than in the control group (p<0.001 for all these parameters). In the receiver operating characteristic curve analysis, the cut-off value for NLR and PLR for the presence of *H. pylori* was calculated as ≥2.39 [sensitivity: 67.3%, specificity: 79.4%, area under the curve (AUC): 0.747 (0.637-0.856), p<0.0001] and ≥133.3 [sensitivity: 61.8%, specificity: 55.9%, AUC: 0.572 (0.447-0.697), p<0.05], respectively.

Conclusion: The present study shows that NLR and PLR are associated with *H. pylori* positivity based on ^{14}C-UBT, and they can be used as an additional biomarker for supporting the ^{14}C-UBT results.

Keywords: ^{14}C urea breath test, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio

Öz

Amaç: Nötrofil/lenfosit oranı (NLR) ve trombosit/lenfosit oranı (PLR), çeşitli hastalıklarda enflamatuvar bir belirteç olarak kullanılmaktadır. Buna karşın, *Helicobacter pylori* tanısında NLR ve PLR'nin kullanılabilirliği ile ilgili çok az veri vardır. Bu çalışmada *H. pylori* tanısında ^{14}C üre nefes testi (^{14}C-UBT) sonuçları ile NLR ve PLR arasındaki ilişkiyi değerlendirmeyi amaçladık.

Yöntemler: Retrospektif olarak toplam 89 hastanın sonuçları incelendi. ^{14}C-UBT sonuçlarına göre, hastalar *H. pylori* (+) ve *H. pylori* (-) olarak iki gruba ayrıldı. İki grup; hemoglobin, beyaz kan hücresi (WBC) sayısı, nötrofil sayısı, lenfosit sayısı, NLR, trombosit sayısı ve PLR'yi içeren hematolojik parametreler yönünden karşılaştırıldı.

Bulgular: *H. pylori* (+) hastalarda; toplam WBC sayısı, nötrofil sayısı, NLR ve PLR, kontrol grubuna göre anlamlı derecede yüksekti (tüm bu parametreler için; p<0,001). Alıcı işletim karakteristiği eğrisi analizinde, *H. pylori* varlığı için NLR ve PLR eşik değerleri sırasıyla, ≥2,39 [duyarlılık: %67,3; özgüllük: %79,4; eğri altında kalan alan (AUC): 0,747 (0,637-0,856) p<0,0001] ve ≥133,3 [duyarlılık: %61,8; özgüllük: %55,9; AUC: 0,572 (0,447-0,697); p<0,05] olarak hesaplandı.

Sonuç: Bu çalışma, *H. pylori* pozitifliği ile NLR ve PLR'nin ilişkili olduğunu ve ^{14}C-UBT sonuçlarını desteklemek için bunların ek bir biyolojik belirteç olarak kullanılabileceğini göstermiştir.

Anahtar Sözcükler: ^{14}C üre nefes testi, nötrofil/lenfosit oranı, trombosit/lenfosit oranı

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Introduction

Helicobacter pylori is a bacterium which is common throughout the world. It plays a role in the pathogenesis of some upper gastrointestinal diseases, such as gastritis, peptic ulcer disease (PUD), gastric cancer (GC) and MALT lymphoma. *H. pylori* is more frequent and acquired at an earlier age in developing countries compared with developed countries (1).

Accurate detection of *H. pylori* is very important for the treatment of *H. pylori* infection. If it is not treated, infection persists and can result in chronic inflammation in the gastric mucosa, which may lead to the development of pathological conditions including PUD and GC (2,3).

Nowadays, several noninvasive-invasive diagnostic methods are used for the detection of *H. pylori.* ^{14}C urea breath test (^{14}C-UBT) has been used as a noninvasive method for the diagnosis of *H. pylori.* In this test, gastric urease activity is detected by measuring isotopic CO_2 excretion in breath after oral administration of ^{14}C-urea.

There are various recommendations for the best diagnostic testing for *H. pylori* by related institutions and study groups, but the choice of these diagnostic methods usually depends on clinical circumstances (4,5). On the other hand, these diagnostic methods have advantages and limitations over each other.

Neutrophil/lymphocyte ratio (NLR), obtained by dividing neutrophil count by lymphocyte count, is a novel laboratory marker to determine systemic inflammation and it is being measured routinely in peripheral blood (6).

In recent years, numerous clinical studies have revealed that there was an association between NLR and several chronic diseases such as diabetes, hypertension, atherogenesis, and other inflammatory disorders. Many studies suggest that NLR may be diagnostically useful for various systemic diseases (7,8). Also, platelet-to-lymphocyte ratio (PLR) is suggested to be a recent hematological parameter indicating the inflammatory and prothrombotic state.

However, there are only a few studies about the relationship between *H. pylori* and NLR in the literature. To our knowledge, there is not any study about the relationship between *H. pylori* and PLR. Therefore, we aimed to investigate the association between ^{14}C-UBT results and NLR, PLR and the other haematological parameters in *H. pylori* diagnosis.

Methods

Study Population and Design

In our study, we retrospectively evaluated records of 89 patients (55 females, 34 males) with dyspepsia who were admitted to the gastroenterology outpatient clinic and then referred to the nuclear medicine department at a university hospital for ^{14}C-UBT.

Exclusion criteria were pregnancy, age <18 years, gastrointestinal disease, surgery to the gastrointestinal tract, comorbidities such as diabetes mellitus, hepatic, renal and cardiovascular diseases (CVDs), abnormal thyroid function tests, previous history of local or systemic infection, acute-chronic inflammatory or autoimmune disease, use of any medication such as corticosteroids for inflammatory condition and hematologic malignancy, use of proton pump inhibitors (PPIs), histamine H2 receptor antagonists, antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) in the past three months.

According to the ^{14}C-UBT results, the patients were divided into two groups: *H. pylori*-positive [*H. pylori* (+)], *H. pylori* -negative [*H. pylori* (-)]. Patients with *H. pylori* (-) results were considered as control group. We compared the haematological parameters, including hemoglobine (Hb), total white blood cell (WBC), neutrophil, lymphocyte, platelet counts, NLR and PLR values, between the gorups.

Evaluation of *Helicobacter pylori* infection

H. pylori infection was diagnosed using the ^{14}C-UBT (Heliprobe® System, Kibion, Uppsala, Sweden) in all patients. ^{14}C-UBT was performed after an overnight fast and at least two months without antibiotics, PPIs, histamine H2 receptor or NSAIDs therapy. Capsules containing 37 kBq (1 μCi) ^{14}C with urea/citric acid (Helicap) were swallowed by the patients with 25 mL of water. Breath samples of patients were collected with a special dry cartridge system (Heliprobe BreathCard) at 10 min. The Heliprobe BreathCard was inserted into a Geiger-Muller counter (Heliprobe analyzer) and activity counted for 250 s. Test results were specified both as counts per minute (cpm) and as grade (0: not infected, cpm <25; 1: equivocal, cpm 25–50; 2: infected, cpm >50).

Peripheral Blood Sampling and Analysis

Five millilitres of peripheral venous blood were collected from each patient in ethylenediaminetetraacetic acid-containing and gel Vacutainer tubes. Complete blood count (CBC) was examined by an automated hematology analyzer system (ABX Pentra DF 120, Horiba Medical, United Kingdom) in our center.

The total WBC count, neutrophil count, lymphocyte count, platelet count and Hb values were recorded. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Similarly, PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count.

Statistical Analysis

All statistical analyses were performed using the SPSS statistical software package, version 18.0 (SPSS Inc.,

Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation, and categorical data were reported as percentages. The Student's t-test was used to compare continuous parametric variables. The chi-square test was used to compare distributions of categorical variables. The cut-off values for NLR and PLR for the prediction of *H. pylori* (+) and their respective sensitivity and specificity values were estimated using receiving operating characteristic (ROC) curve analysis. A p value of less than 0.05 was considered statistically significant.

Results

A total of 89 patients with a mean age of 47.1±12.4 years (range: 19-69,) were included in the study. Fifty five (61.8%) patients were female and 34 (38.2%) were male.

The number of *H. pylori* (+) and control group patients was 52 (58.4%) and 37 (41.6%), respectively. The mean age of the patients in the *H. pylori* (+) and control groups was 46.4±13.1 years and 47.8±11.8 years, respectively. The *H. pylori* (+) group consisted of 30 female and 22 male patients while there were 25 female and 12 male patients in the control group. There was no statistically significant difference in age and gender between the two groups (p>0.05).

We compared haematological parameters i.e. Hb, WBC count, neutrophil count, lymphocyte count, NLR, platelet count and PLR between the gorups. The mean total WBC count, neutrophil count, NLR and PLR in *H. pylori* (+) patients were significantly higher than in the control group (p<0.001, p<0.001, p<0.001 and p=0.032, respectively). No significant difference was noted in lymphocyte counts between the groups (p>0.05).

In addition, there was no significant difference in other haematological parameters (Hb and platelet count) between the groups (p>0.05 for all compared parameters).

Baseline clinical and laboratory parameters and demographic characteristics of patients are summarized in Table 1.

Additionally, ROC curve analysis was used to determine the optimum cut-off levels of the NLR and PLR in association with *H. pylori* positivity.

In the ROC curve analysis, an NLR level cutoff point of more than 2.39 predicted the presence of *H. pylori* with a sensitivity of 67.3% and specificity of 79.4% [ROC area under curve: 0.747, 95% confidence interval (CI): 0.637-0.856, p<0.0001; Figure 1].

In the ROC curve analysis, a PLR level cutoff point of more than 133.3 predicted the presence of *H. pylori* with a sensitivity of 61.8% and specificity of 55.9% (ROC area under curve: 0.572, 95% CI: 0.447-0.697, p<0.05; Figure 1).

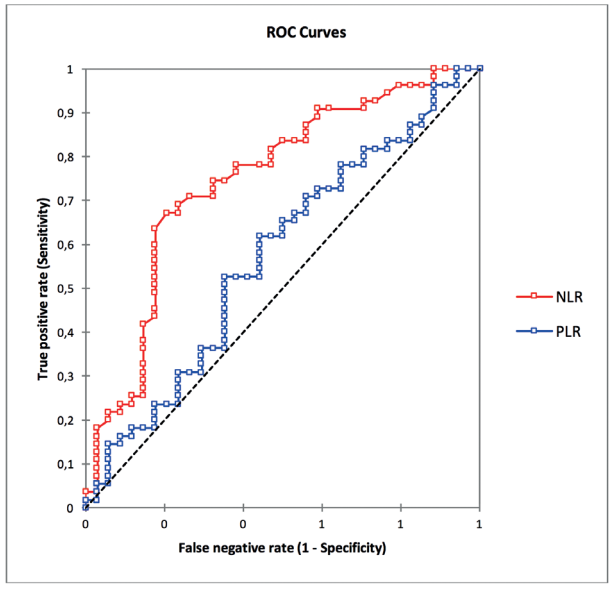

Figure 1. The receiver-operating characteristic curve analysis of neutrophil/lymphocyte ratio and platelet/lymphocyte ratio for predicting *Helicobacter pylori* positivity
ROC: Receiver-operating characteristic, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio

Table 1. Baseline clinical and laboratory parameters and demographic characteristics of patients

	Helicobacter pylori (+) (n=52)	*Helicobacter pylori* (-) (n=37)	p value	All (n=89)
Age (mean-SD), years	46.4±13.1	47.8±11.8	p>0.05	47.1±12.4
Gender (male/female)	22/30	12/25	p>0.05	34/55
Hb (g/dL) (mean-SD)	13.82±1.05	14.13±0.93	p>0.05	14.08±0.98
WBC (/mm³) (mean-SD)	6757±1120	6039±839	p<0.05	6483±1075
Neutrophil (/mm³) (mean-SD)	4269±883	3407±576	p<0.05	3905±884
Lymphocyte (/mm³) (mean-SD)	1591±306	1671±285	p>0.05	1622±299
NLR (mean-SD)	2.72±0.48	2.05±0.23	p<0.05	2.47±0.52
Platelet (/mm³) (mean-SD)	228063±43409	213867±38881	p>0.05	222640±42087
PLR (mean-SD)	148.3±39.9	132.4±37.2	p<0.05	142.3±39.5

Hb: Hemoglobin, WBC: White blood cell, NLR: Neutrophil/lymphocyte ratio, PLR: Platelet/lymphocyte ratio, SD: Standart deviation

Discussion

We know that the components of complete CBC, such as WBC count, neutrophil, lymphocyte, NLR, platelet and PLR, can be used as a predictor in many diseases related with inflammatory reactions. Especially NLR and PLR are novel prognostic and inflammatory markers in patients with cancer as well as in those with inflammatory diseases and CVDs. These markers are inexpensive, simple and relatively effective tools for the diagnosis and for predicting the prognosis of several diseases.

Lymphocytopenia is a common finding of chronic inflammation. The reasons for this situation are increased lymphocyte apoptosis and a shift towards increasing neutrophils and decreasing lymphocytes in the leukocyte production of bone marrow. Lymphocytes represent a more convenient immune response, while neutrophils cause a destructive inflammatory reaction (7). Recently, numerous epidemiological and clinical studies have shown an association between leukocyte counts and several diseases, and they suggest that peripheral leukocyte counts and NLR may be diagnostically useful (8).

As far as is known, *H. pylori* leads to accumulation of neutrophils and lymphocytes in the gastric mucosa, and causes local chronic inflammation if not eradicated. This local inflammation may initiate a systematic response in the host. *H. pylori* is suggested to be associated with low-grade inflammation (9). Proinflammatory cytokines, such as tumor necrosis factor, interleukin (IL)-1 and IL-6, stimulate the generation of leukocytes from bone marrow stem cells (10). In their study, Romero-Adrian et al. (11) reported an increase in the production of these cytokines in *H. pylori* -infected persons.

In our study, NLR was calculated and evaluated in both groups, and a higher WBC count, neutrophil count and NLR were observed in the *H. pylori* (+) group compared to that in the control group.

These results are consistent with the literature and indicate that there is an increase in neutrophil count, there is no significant change in lymphocyte count. As mentioned in the literature, the reason for this situation may be the increase in the expression of IL-17A that has been shown to increase neutrophil counts via induction of granulocyte colony stimulating factor, in *H. pylori*-infected persons (12-14).

Several studies have demonstrated the relationship between *H. pylori* and systemic diseases. The relevant researches have shown that *H. pylori* induces systemic inflammation and adversely affects absorption of nutrients, so that increasing the risk of several diseases such as CVD, stroke, anemia, glaucoma, Alzheimer's disease, rosacea, eczema, chronic hives, diabetes, thyroid disease, and idiopathic thrombocytopenic purpura (15-20). As mentioned before, the variation of WBC subtypes may be seen frequently in these diseases. For this reason, the records of the patients included in our study were examined carefully and those identified as having these diseases were excluded. On the other hand, Proctor et al. (21) stated that absolute counts of neutrophil and lymphocyte might change with various physiological, pathological and physical conditions, but NLR is not affected by these factors. In agreement with this study, we also think that NLR values were more valuable for the prediction of *H. pylori* infection; although the mean total WBC count, neutrophil count and NLR were found to be significantly higher in the *H. pylori* (+) patients. However, there are few studies on the subject in the literature; the relationship between NLR and *H. pylori* still need to be investigated with further studies.

Similarly, PLR is a recent hematological parameter indicating the inflammatory and prothrombotic state. Recently in numerous studies (22-26), PLR is suggested to be a novel prothrombotic and inflammatory marker in some heart diseases and cancers, however, the relationship between PLR and *H. pylori* has not been investigated. To the best of our knowledge, this is the first study investigating PLR in *H. pylori* disease. In the present study, PLR was calculated and assessed in both groups, and it was found that PLR was significantly higher in the *H. pylori* (+) group than in the control group. These results show that PLR values may also lead the way to *H. pylori* diagnosis as NLR, but further studies are needed.

Besides the diagnostic value of NLR and PLR, numerous recent studies (22-26) have demonstrated that they were markers of prognosis in several diseases.

In the light of our results and related prior studies, we assume that it is more convenient to use PLR and NLR values together for *H. pylori*, instead of using them seperately.

Currently, there is not a known cut-off value for NLR and PLR for predicting clinical diagnosis or outcomes in a variety of diseases. Some studies show that a high threshold of NLR is found in cancer patients. We found that the cut-off value for NLR for predicting *H. pylori* was lower than in cancer-related studies. The reason for this situation can be considered that the cancer-related systemic inflammatory response is associated with alternation in circulating WBCs, specifically with the presence of neutrophilia with a relative lymphocytopenia (27).

As a result, it can be suggested that NLR and PLR can not be used as a single marker for the diagnosis of *H. pylori*; but they might be used to predict the *H. pylori* positivity, since they are cheap, simple and routinely used tests in daily clinical practice.

Study Limitations

Our study has some limitations. These can be listed as follows: i) the study was a retrospective analysis which was the main limitation, ii) the number of patients in the study and control groups was small, iii) single blood sampling values were used rather than follow-up values, iv) the patient follow-up data were absent after the eradication therapy, v) we did not check responses to the therapy and variation of this haematological parameters over time vi) and, we did not compare the findings with other inflammatory markers.

Conclusion

NLR and PLR are inexpensive, simple and, a novel prognostic and inflammatory markers for the diagnosis and prognosis of several diseases. Therefore, we evaluated NLR and PLR values for *H. pylori* in this study. We found that NLR and PLR were significantly increased in *H. pylori* (+) patients based on ^{14}C-UBT. We suggest that NLR and PLR can be used as an additional biomarker for supporting the ^{14}C-UBT results.

Ethics

Ethics Committee Approval: Retrospective study.
Informed Consent: Retrospective study.
Peer-review: Internally peer-reviewed.
Authorship Contributions

Surgical and Medical Practices: E.Ş. Concept: E.Ş. Design: E.Ş. Data Collection or Processing: E.Ş., U.E. Analysis or Interpretation: E.Ş. Literature Search: E.Ş., U.E. Writing: E.Ş.

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