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Evaluation of Platelet-to-Lymphocyte Ratio and 13C-Urea Breath Test as Non-Invasive Markers for Helicobacter pylori Infection

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ABSTRACT

Background: *Helicobacter pylori (H. pylori)* is a widespread stomach pathogen known to cause chronic gastritis, peptic ulcers, and gastric cancer. Reliable diagnosis and measurement of infection severity are crucial for proper treatment.

Aim: This study aimed to analyze blood-based and inflammatory markers in patients tested for *H. pylori* and investigate the potential of the platelet-to-lymphocyte ratio (PLR) as a simple, non-invasive diagnostic tool.

Methods: We divided 110 patients into three groups based on test results: Group I (n = 27) tested negative in blood, stool, and urea breath test (UBT); Group II (n = 34) was positive only in UBT (C13 > 4%); and Group III (n = 49) tested positive in all three tests. We compared blood cell counts, including white blood cells (WBC), hematocrit (HCT), lymphocytes, platelets, and PLR. The diagnostic performance of PLR was evaluated using ROC curve analysis.

Results: WBC, HCT, and lymphocyte counts showed no significant differences between groups. However, platelet counts were notably higher in Group III than in Groups I and II ($p < 0.05$). UBT (C13%) values rose progressively from Group I to Group III, indicating increased bacterial activity. PLR was significantly higher in Group III compared to Group II ($p < 0.05$). ROC analysis confirmed PLR's strong diagnostic ability, with an AUC of 97%, 95% sensitivity, 100% specificity, and an optimal cutoff value of 9.5.

Conclusion: PLR proved to be a highly accurate, affordable, and non-invasive marker for active *H. pylori* infection. UBT (C13%) levels also correlated with infection severity, supporting its use in detecting and measuring bacterial load. These findings suggest PLR could be a valuable tool in clinical practice.

Keywords: *Helicobacter pylori (H. pylori)*, non-invasive, platelets-to-lymphocytes ratio (PLR), urea breath test (UBT)

1. Introduction

A gram-negative, Helicobacter pylori (*H. pylori*) is a microaerophilic bacterium that inhabits the human stomach, is a well-established pathogen related with multiple gastrointestinal diseases or disorders. These include, gastric adenocarcinoma, chronic gastritis, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and peptic ulcer disease. Globally, *H. pylori* infect nearby half of the world's population (1). Recent epidemiological data indicate a decline in adult prevalence from 52.6% (pre-1990) to 43.9% (2015-2022), while pediatric rates remained stable at 35.1% during the same period (2).

Regional studies in Iraq demonstrate varying infection rates. In Duhok, serological testing revealed *H. pylori* IgG antibodies in 40.02% of adults (2018-2020) (3). Similarly, in Sulaimani, 54.9% of patients undergoing urea breath testing (UBT) were positive (4), while Erbil reported a 53.3% prevalence in 2019 (5). These findings underscore the persistent public health burden of *H. pylori* in the region.

Accurate diagnosis is critical for preventing disease progression and improving clinical outcomes. Diagnostic approaches include both invasive (endoscopic biopsy with histology/rapid urease testing) and non-invasive methods (serology, stool antigen tests, UBT) test (6). While serology is cost-effective, it cannot distinguish active from past infections due to prolonged antibody persistence (7). Stool antigen tests detect current infection but are influenced by recent antibiotic or proton pump inhibitor use (8). In contrast, the 13C-UBT offers high specificity and sensitivity by directly measuring *H. pylori* urease activity (9).

Emerging evidence suggests that hematologic markers, particularly the platelet-to-lymphocyte ratio (PLR), may reflect systemic inflammation during *H. pylori* infection. Platelets increase during inflammatory states, while lymphocytes often decrease in chronic inflammation. Elevated PLR has been linked to various pathologies, including cancer and cardiovascular disease (10, 11). Still, its role in *H. pylori* infection, especially in relation to UBT results and bacterial activity, remains underexplored.

This study investigated the association between *H. pylori* diagnostic test results and hematologic inflammatory markers (platelet count, PLR). By integrating routine blood parameters with non-invasive testing, we aimed to enhance clinical assessment of infection severity and support personalized treatment strategies. Such an approach could improve diagnostic precision and optimize therapeutic decision-making for *H. pylori*-associated diseases.

2. METHODS AND MATERIALS

This study is retrospective and included 110 patients with H. pylori (male=59 and female=51). The average age in males was 35.9 years (minimum ages = 12 and maximum = 83 years), while among females it was 35.1 years (minimum ages = 9 and maximum = 81 years). The diagnostic examination for the patients included serological, stool tests for antigen, and a urea breath test (UBT), along with invasive examination (endoscopy and histological) (12). Patients with endoscopy or histologically negative findings were excluded.

According to the results, the patients were divided into three different groups. The patients with all tests negative were considered as the first group (group I, n=27), the second group (group II, n=34) included only

UBT positive (the tests for serum and stool antigen were negative), the third group (group III, n=49) was all tests positive. Hematological tests were also done for all patients and included total white blood cells (WBC), absolute lymphocytes, hematocrits (HCT), and platelets (PLT).

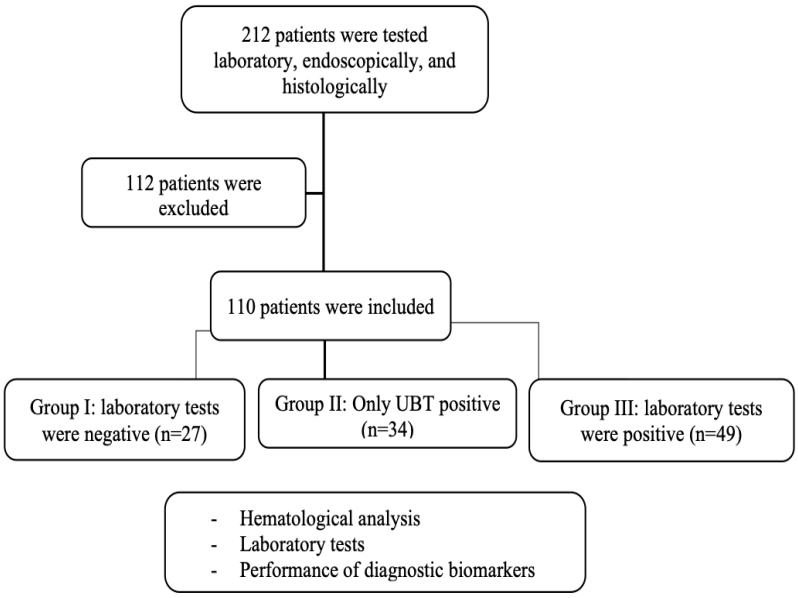

Figure 1: Flowchart diagram of studied participants

2.1. Laboratory investigation

Serological testing was performed using the OnSite H. pylori Ab Combo Rapid Test (CTK Biotech, Cat. R0191C, USA), which is a qualitative sandwich lateral flow chromatographic immunoassay designed to detect *H. pylori*-specific antibodies (IgG, IgM, and IgA) in human serum, plasma, or whole blood, following the manufacturer's protocol (13).

For stool antigen detection, the OnSite H. pylori Ag Rapid Test (CTK Biotech, Cat. R0192C, USA) was employed. This lateral flow chromatographic immunoassay qualitatively identifies *H. pylori* antigens in fecal samples, as per standardized procedures (13, 14).

Additionally, the urea breath test (UBT) was conducted to assess urease activity. This test measures the enzymatic conversion of ingested 13C-labeled urea into $13CO_2$ and ammonia, with subsequent detection of $13CO_2$ in exhaled breath, confirming active *H. pylori* infection.

2.2. Statistical analysis

The data were analyzed to statistical data analysis (GraphPad Prism 10). The normality distribution of the data was analyzed using the Shapiro-Wilk test (15). The one-way ANOVA were used for analyzing normally distributed data, while the Kruskal-Wallis test was used for analyzing non-parametric data. The accuracy of the diagnostic parameters was sat by the Area under the ROC curve (AUC). The optimal cutoff points of UBT were identified based on the specificity and sensitivity for the discrimination of H. pylori patients for disease activity

3. RESULTS

The analysis revealed no significant difference in white blood cell (WBC) counts between *H. pylori*-positive and negative participants ($p = 0.056$, Figure 1A). The median WBC values were 7±0.57 (positive) versus

5±0.41 (negative). Platelet counts showed significant elevation (p = 0.05) in patients positive across all three diagnostic tests (serology, stool antigen, and UBT; 315.6±38.14) compared to negative controls (243.3±64.93). However, participants with only UBT positivity (275.4±70.39) showed no significant difference (Figure 1B). Lymphocyte levels remained comparable across all groups (Figure 1C): negative controls (1.62±1.16), UBT-only positive (1.23±0.77), and triple-positive patients (1.68±0.73). Similarly, hematocrit (HCT) values showed no significant intergroup variation (p = 0.98 and 0.9, Figure 1D), with means of 37.86±5.58 (negative), 38.29±6.04 (UBT-only), and 38.89±5 (triple-positive).

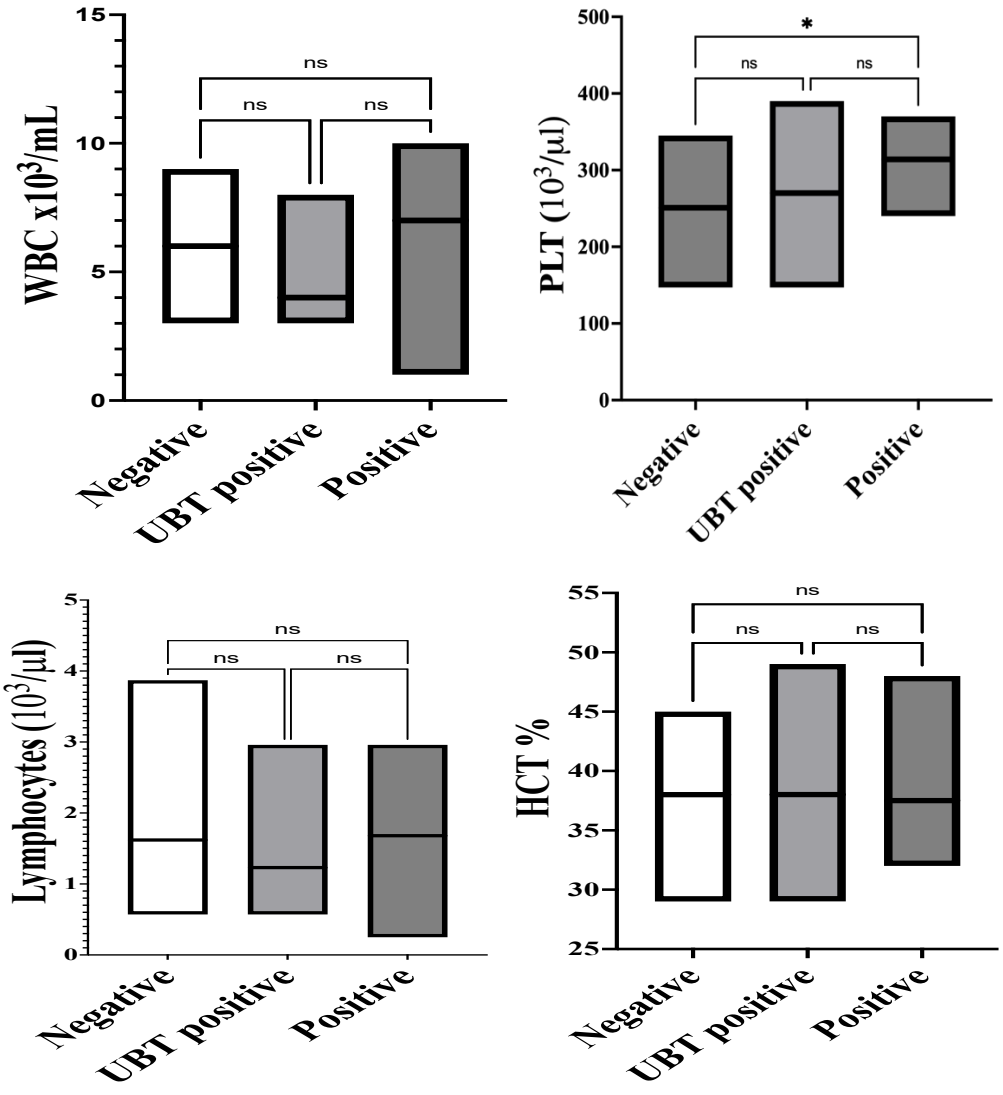

Figure 2: Hematological results across groups, A. the level of WBCs, B. the platelet levels, C. the lymphocyte levels, and D. the Hematocrit (HCT%) levels.

ns: indicate statistical non-significance.

*: indicate the statistical difference at the level of significance p=0.05

UBT test results

Based on international standards, a C13 Urea Breath Test (UBT) value ≥4% indicates *H. pylori* positivity (16). Our findings revealed that among 56 patients negative by both serology and stool antigen tests, 34 (60.7%) showed UBT positivity (mean=7.56% ± 3.22). Comparative analysis demonstrated significantly

higher UBT values in fully positive patients (10.58% ± 0.32). full negative group (6.6% ± 0.33; p=0.0001), UBT-only positive cases (7.56% ± 3.22). As shown in Figure 2, triple-negative patients exhibited significantly lower UBT values than both UBT-positive and fully positive groups (p<0.05), confirming the test's discriminative capacity for active infection.

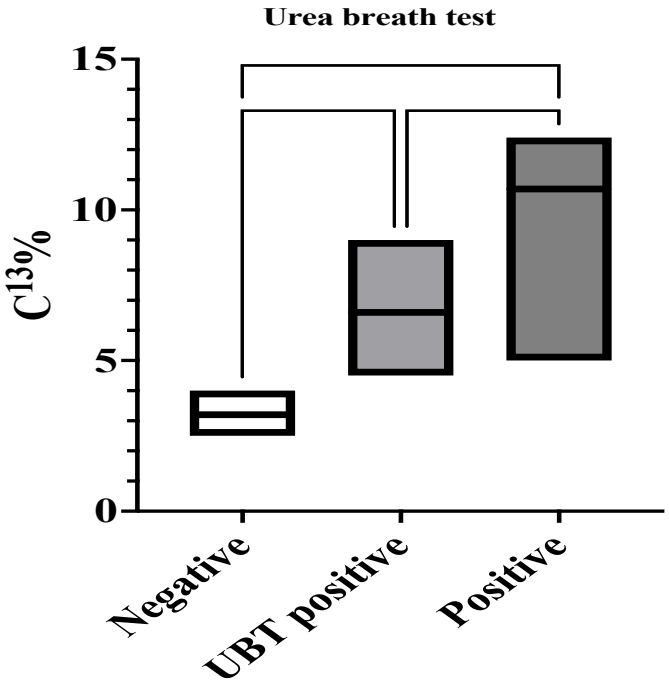

Figure 3: The percentage of C13 concentration in participants for estimation of UBT.

*, **, and ****: indicate the statistical difference at the level of significance p=0.05, 0.01, and 0.0001, respectively

Platelet-to-lymphocyte ratio

To evaluate infection severity, we calculated the platelet-to-lymphocyte ratio (PLR). Our analysis revealed significantly elevated PLR values in patients with confirmed H. pylori infection (positive across all diagnostic tests) compared to those with only UBT positivity (p=0.05). This finding suggests that PLR may serve as a potential marker for assessing the inflammatory burden associated with H. pylori infection.

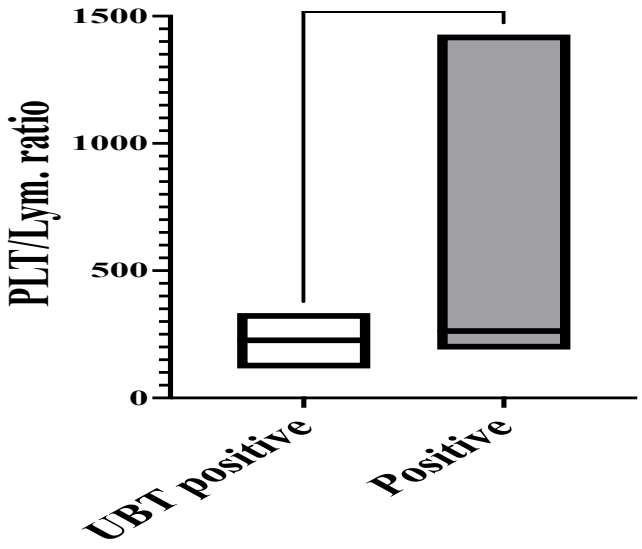

Figure 4: Platelets-to-lymphocytes ratio among patients with only UBT positive and patients with serological, stool antigen, and UBT positive.

*: indicates the statistical difference at the level of significance p=0.05.

Performance of Diagnostic Biomarkers

The present study found several leukocyte ratio markers that may distinguish between group II and III during the period of disease. ROC studies showed that the PLR ratio (AUC 0.97, sensitivity 95%, specificity 100%, and 95% CI: 76.39% to 99.74%) was a good biomarker to distinguish between patients infected with H. pylori who were only UBT positive and all positive markers with the threshold of 9.5 (Figure 4).

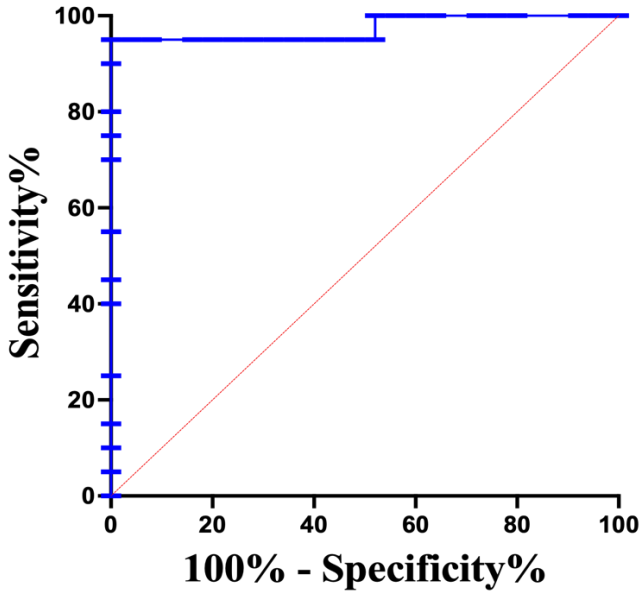

Figure 5: Receiver operating characteristic (ROC) curve of platelets-to-lymphocytes ratio in predicting disease level.

4. Discussion

This investigation sought to analyze and contrast laboratory parameters undergoing evaluation for H. pylori infection through multiple diagnostic approaches: serum antibody detection, fecal antigen testing, and 13C-urea breath testing. Initial analysis revealed that standard hematological measures, including white blood cell counts, hematocrit levels, and absolute lymphocyte counts, showed no statistically significant differences between the study groups. These observations imply that routine hematologic parameters possess limited diagnostic value for detecting H. pylori infection or assessing its clinical severity. Our findings align with existing research demonstrating that while such markers may indicate systemic inflammatory responses or hematologic abnormalities, they demonstrate poor specificity for identifying localized gastric infections in the absence of systemic manifestations (17).

Our analysis revealed a statistically significant increase in platelet counts among Group III patients compared to both Group I and Group II. This observation aligns with current medical literature that identifies platelet counts as reliable indicators of systemic inflammatory processes (18). The thrombocytosis observed in patients with confirmed H. pylori infection through multiple diagnostic methods likely represents chronic gastric inflammation resulting from persistent bacterial colonization and mucosal damage associated with long-term infection. The underlying mechanism appears to involve H. pylori-induced cytokine production (particularly IL-1, IL-6, and TNF-α), which stimulates platelet production in the bone marrow (19). These findings position platelet count as a potentially valuable, albeit indirect, biomarker for

assessing infection severity, inflammatory burden, and disease chronicity in H. pylori-positive patients. The correlation between multi-test positivity and elevated platelet levels strengthens the clinical relevance of this hematologic parameter in infection monitoring and management.

Notably, 13C-urea breath test (C13%) values demonstrated significant elevation in Group III versus Groups I and II, with Group II also showing higher values than Group I. As a validated non-invasive technique, UBT quantitatively assesses active urease production (8). The graduated rise in C13% across groups likely indicates either greater bacterial colonization or heightened urease expression. These findings confirm UBT's dual role in both infection diagnosis and severity assessment. The strong C13% elevation in triple-positive cases particularly underscores the relationship between urease activity and multi-test concordance.

The platelet-to-lymphocyte ratio (PLR), a combined inflammatory index assessing both thrombocyte and lymphocyte responses, showed marked elevation in Group III versus Group II. This composite biomarker has gained recognition across infectious diseases, cancers, and autoimmune disorders as an indicator of systemic inflammation (20). For H. pylori infections, increased PLR values potentially signify either heightened immune activation or more severe gastric inflammation. The substantially greater PLR observed in triple-test-positive patients strengthens the link between multi-method diagnostic positivity and whole-body inflammatory status. As chronic inflammation typically suppresses lymphocytes while boosting platelets, elevated PLR values likely capture both components of this pathological imbalance (18, 20).

Furthermore, PLR demonstrated exceptional diagnostic accuracy in our investigation. ROC curve analysis revealed an AUC of 97%, confirming outstanding discriminatory power. The identified optimal PLR cutoff of 9.5 achieved 95% sensitivity and perfect 100% specificity. These results underscore PLR's promise as an affordable, non-invasive supplementary test for H. pylori detection, especially valuable in low-resource areas lacking endoscopic capabilities. The remarkable specificity indicates that PLR values exceeding this threshold reliably predict active infection with negligible false-positive results.

Pathophysiologically, H. pylori's interaction with blood parameters involves complex mechanisms. Persistent infection triggers cytokine-mediated bone marrow activation, enhancing platelet production while causing lymphocyte depletion through sustained antigen exposure and immune fatigue. Consequently, PLR serves as both an inflammatory indicator and a marker of prolonged bacterial colonization (18). This application proves especially valuable amid rising antibiotic-resistant H. pylori cases, enabling improved patient classification for precision therapy.

Diagnostic limitations and method interactions warrant careful consideration. Serology often yields prolonged positivity post-treatment, reducing its reliability for active infection detection **(21)**. Stool antigen tests demonstrate good sensitivity but are vulnerable to collection techniques, recent PPI/antibiotic use, and irregular pathogen excretion (22). While UBT represents the gold standard for active infection, rare false positives may occur from non-H. pylori urease producers (23). The consistent triple-positive results in Group III strengthen diagnostic certainty and enable comprehensive biomarker assessment.

This investigation possesses notable strengths, particularly its utilization of multiple diagnostic approaches and integration of inflammatory biomarkers for comprehensive comparison. Nevertheless, certain limitations

exist. The sample size remains somewhat limited, especially for subgroup analyses, potentially impacting statistical robustness. Furthermore, the absence of endoscopic verification or histopathological assessment in the study design precluded additional correlations with gastric mucosal inflammation and precise infection severity grading.

5. Conclusion

In summary, our results show that triple-positive H. pylori patients (serum, stool, and UBT) displayed significantly higher platelet counts and PLR values, suggesting enhanced systemic inflammation. UBT C13% levels progressively increased across diagnostic groups, potentially mirroring bacterial burden or urease activity. PLR proved particularly valuable among hematologic markers, demonstrating excellent sensitivity and specificity for infection detection. These findings position PLR and quantitative UBT as clinically useful, non-invasive methods for both diagnosing H. pylori and evaluating infection severity.

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