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Association of Helicobacter pylori and Intestinal Parasitic Infections with Anaemia among Dyspeptic Patients in Benghazi, Libya: A Cross-Sectional Study

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A B S T R A C T

Background and objectives. *Helicobacter pylori* and intestinal parasites are both common in low- and middle-income settings, and each can lower haemoglobin through blood loss, malabsorption, or chronic inflammation. While both pathogens are prevalent, their concurrent presence and association with haematological parameters among symptomatic patients require further clarification. This study aimed to investigate the prevalence of *H. pylori* and intestinal parasitic infections among adult dyspeptic patients in Benghazi, Libya, and to evaluate their association with haematological parameters and anaemia.

Methods. A hospital-based cross-sectional study was carried out at Benghazi Medical Centre among 304 adults systematically selected from outpatient gastroenterology referrals for investigation of persistent dyspepsia. A stool sample from each patient was tested for *H. pylori* antigen using a commercial enzyme-linked immunosorbent assay (ELISA) kit, examined microscopically for parasites by direct wet mount and formol-ether sedimentation, and screened for faecal occult blood. Complete blood counts were obtained on an automated haematology analyser (Sysmex KX-21N) within 2 hours of blood collection. Multivariable binary logistic regression was performed to adjust for potential confounders such as age and sex, reporting Adjusted Odds Ratios (aOR) with 95% Confidence Intervals (CI). Proportions were compared with the chi-square test and means with independent-samples t-tests.

Results. *H. pylori* antigen was detected in 89 of 304 patients (29.3%). Within this group, 15 patients (16.9%) carried *Giardia lamblia*, 7 (7.9%) *Entamoeba histolytica*, and 56 (62.9%) had a positive faecal occult blood test. Anaemia was present in 80 of the 89 infected patients (89.9%) compared with 3 of 215 uninfected patients (1.4%; $p < 0.001$). On multivariable logistic regression, *H. pylori* infection remained independently associated with a significantly higher likelihood of anaemia (aOR = 210.5, 95% CI: 58.2–761.4, $p < 0.001$). Mean haemoglobin (9.5 ± 2.0 vs 12.3 ± 1.4 g/dL, $p < 0.001$), haematocrit (29.1 ± 5.9 vs 36.3 ± 4.3%, $p < 0.001$), red cell count (3.8 ± 0.82 vs 4.3 ± 0.4 × 1012/L, $p = 0.009$), and platelet count (138.8 ± 53.5 vs 282.7 ± 54.4 × 109/L, $p < 0.001$) were all lower in infected patients, while the white cell count was higher (10.6 ± 3.6 vs 5.9 ± 1.5 × 109/L, $p < 0.001$).

Conclusions. In this clinic population, *H. pylori* infection was strongly associated with anaemia, lower red cell parameters, and alterations in white cell and platelet counts. A substantial minority of infected patients carried intestinal protozoa. Given the cross-sectional study design and small co-infected subgroups, these findings demonstrate significant clinical associations rather than direct synergistic causality. Integrated screening for gastric and intestinal pathogens in dyspeptic patients with anaemia is recommended.

ارتباط عدوى الملوية البوابية والطفيليات المعوية بالمعايير الدموية وفقر الدم بين مرضى عسر الهضم في بنغازي، ليبيا: دراسة مقطعية

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الملخص

الخلفية والأهداف: تُعدّ جرثومة الملوية البوابية (جرثومة المعدة) والطفيليات المعوية شائعة في البلدان ذات الدخل المنخفض والمتوسط، ويمكن لكلٍّ منهما أن يُخفّض مستوى الهيموجلوبين من خلال فقدان الدم، أو سوء الامتصاص، أو الالتهاب المزمن. ورغم شيوع كلا العاملين الممرضين، فإن وجودهما المتزامن وارتباطهما بالمعايير الدموية لدى المرضى الذين يعانون من أعراض يتطلب مزيدًا من التوضيح. هدفت هذه الدراسة إلى التحقق من مدى انتشار جرثومة الملوية البوابية والعدوى الطفيلية المعوية بين مرضى عسر

الكلمات المفتاحية
الملوية البوابية
الطفيليات المعوية
فقر الدم؛ المعايير الدموية
العدوى المشتركة

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الهضم البالغين في بنغازي، ليبيا، وتقييم ارتباطهما بالمعايير الدموية وفقر الدم.

المنهجية وطرق البحث: أُجريت دراسة مقطعية في مستشفى مركز بنغازي الطبي، شملت 304 من المرضى بالغين تم اختيارهم بشكل منهجي من بين المرضى المحالين إلى قسم أمراض الجهاز الهضمي الخارجي لفحص عسر الهضم المستمر. تم فحص عينة براز من كل مريض للكشف عن مستضد جرثومة الملوية البوابية باستخدام مجموعة اختبار الإليزا التجارية، وفُحصت مجهريًا للكشف عن الطفيليات عن طريق الفحص المباشر للعينات الرطبة والترسيب بالفورمالين والإيثر، كما تم فحصها للكشف عن الدم الخفي في البراز. أُجريت فحوصات تعداد الدم الكامل باستخدام محلل الدم الآلي (Sysmex KX-21N) في غضون ساعتين من جمع الدم. أُجري تحليل الانحدار اللوجستي الثنائي متعدد المتغيرات لضبط المتغيرات المربكة المحتملة مثل العمر والجنس، وتم الإبلاغ عن نسب الأرجحية المعدلة (aOR) مع فترات ثقة 95%. قورنت النسب باستخدام اختبار مربع كاي، والمتوسطات باستخدام اختبارات t للعينات المستقلة.

النتائج: تم الكشف عن مستضد جرثومة الملوية البوابية لدى 89 من أصل 304 مرضى (29.3%). ضمن هذه المجموعة، كان 15 مريضًا (16.9%) مصابين بجيارديا لامبليا، و7 مرضى (7.9%) مصابين بالأميبا الحالة للنسج، و56 مريضًا (62.9%) كانت نتيجة اختبار الدم الخفي في البراز لديهم إيجابية. وظهر فقر الدم لدى 80 من أصل 89 مريضًا مصابًا (89.9%) مقارنةً بـ 3 من أصل 215 مريضًا غير مصاب (1.4%؛ $p < 0.001$). وباستخدام تحليل الانحدار اللوجستي متعدد المتغيرات، تبين أن الإصابة ببكتيريا الملوية البوابية (H. pylori) مرتبطة بشكل مستقل بزيادة احتمالية الإصابة بفقر الدم بشكل ملحوظ (نسبة الأرجحية المعدلة = 210.5، فاصل الثقة 95%: 58.2-761.4، $p < 0.001$). كان متوسط مستوى الهيموجلوبين (9.5 ± 2.0 مقابل 12.3 ± 1.4 غ/ديسيلتر، $p < 0.001$)، والهيماتوكريت (29.1 ± 5.9 مقابل 36.3 ± 4.3%، $p < 0.001$)، وعدد كريات الدم الحمراء (3.8 ± 0.82 مقابل 4.3 ± 0.4 × 10^{12}/لتر، $p = 0.009$)، وعدد الصفائح الدموية (138.8 ± 53.5 مقابل 282.7 ± 54.4 × 10^9/لتر، $p < 0.001$) أقل لدى المرضى المصابين، بينما كان عدد كريات الدم البيضاء أعلى (10.6 ± 3.6 مقابل 5.9 ± 1.5 × 10^9/لتر، $p < 0.001$).

الاستنتاجات. في هذه الدراسة السريرية، ارتبطت عدوى جرثومة المعدة (H. pylori) ارتباطًا وثيقًا بفقر الدم، وانخفاض مؤشرات تعداد خلايا الدم الحمراء، وتغيرات في تعداد خلايا الدم البيضاء والصفائح الدموية. كما تبين أن نسبة كبيرة من المرضى المصابين يحملون طفيليات معوية. ونظرًا لتصميم الدراسة المقطعية وصغر حجم المجموعات الفرعية المصابة بعدوى مشتركة، فإن هذه النتائج تُظهر ارتباطات سريرية هامة وليست علاقة سببية تآزرية مباشرة. يُوصى بإجراء فحص متكامل للكشف عن مسببات الأمراض المعدية والمعوية لدى مرضى عسر الهضم المصابين بفقر الدم.

الدم الخفي في البراز
عسر الهضم؛ بنغاز
ليبيا

Introduction

Helicobacter pylori is a Gram-negative, spiral, microaerophilic bacterium that colonizes the human gastric mucosa and usually persists for life unless eradicated. It remains one of the most widespread chronic bacterial pathogens worldwide, with high prevalence rates in regions where shared water sources, high population density, and suboptimal sanitation facilitate faecal-oral transmission [1]. Chronic gastric mucosal colonization induces persistent inflammatory responses, laying the foundation for chronic active gastritis, peptic ulceration, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma. Bacterial persistence and chronic mucosal colonization are further complicated by the ability of *H. pylori* to form recalcitrant states and persisted cell populations that resist clearance and sustain long-term low-grade gastric inflammation [2].

Beyond gastrointestinal pathology, *H. pylori* infection has been widely associated with extra gastric manifestations, among which anaemia is prominent [3, 4]. Multiple potential mechanisms explain this association. First, persistent mucosal erosions and micro-ulcerations promote chronic occult gastrointestinal blood loss. Second, loss of parietal cell mass and chronic atrophic gastritis impair gastric acid and ascorbic acid secretion, thereby hindering the reduction of dietary ferric iron (Fe3+) to its absorbable ferrous form (Fe2+). Third, sustained mucosal and systemic inflammatory signalling elevates hepatic hepcidin synthesis, causing iron sequestration within macrophages and enterocytes, mimicking anaemia of chronic disease [5]. In addition, clinical evaluation of hematological and metabolic biomarkers among patient cohorts in Benghazi has highlighted how systemic inflammatory states alter baseline circulating cell lines and metabolic risk profiles [6,7,14,15]. Consensus guidelines recommend testing for and eradicating *H. pylori* in patients presenting with unexplained iron deficiency anaemia [8].

In North Africa and developing regions, this infection burden frequently overlaps with intestinal parasitic infestations and endemic infectious threats [6,9,10,12] Protozoans such as Giardia lamblia and Entamoeba histolytica, as well as soil-transmitted helminths and foodborne protozoan contaminants, share faecal-oral or environmental transmission pathways influenced by sanitation and hygiene practices [11,13]. Parasites adversely impact host haematological status through distinct pathways: G. lamblia causes duodenal villous atrophy and brush-border impairment, reducing nutrient and iron absorption, whereas E. histolytica invades mucosal tissues, causing dysentery and micro-bleeding [11]. When these infections coincide, their combined presence may aggravate host haematological deterioration.

Despite the high burden of gastrointestinal complaints in Libya, published data evaluating concurrent *H. pylori* and parasitic infections and their relationship with measured haematological parameters remain limited. To address this research gap, the current study evaluated the prevalence of *H. pylori* and intestinal parasites among adult patients presenting with persistent dyspepsia at Benghazi Medical Centre, described their distribution by age and sex, and evaluated differences in haematological parameters between infected and uninfected individuals using both univariate and multivariable statistical models.

Materials and Methods

Study Design, Setting and Participants

A hospital-based descriptive cross-sectional study was conducted at Benghazi Medical Centre (BMC), a tertiary referral care hospital in Benghazi, Libya. Adult patients (aged $\geqslant$ 18 years) referred to the gastroenterology outpatient services for investigation of persistent dyspepsia (defined as continuous or recurrent pain or discomfort centered in the

upper abdomen lasting for at least 4 weeks) were eligible for inclusion. Participants were recruited between January and June 2025 using systematic random sampling from the daily clinical referral register (enrolling every third eligible patient).

Patients who had received antibiotics, proton pump inhibitors, bismuth preparations, or antiparasitic medications within the preceding four weeks were excluded, as recent antimicrobial or antisecretory therapy diminishes stool antigen test sensitivity and alters parasitological detection. Patients with known active malignancy, overt upper gastrointestinal bleeding, chronic kidney disease, or prior gastric surgery were also excluded.

The minimum required sample size was calculated using the single proportion formula: $n = (Z2 \times P \times (1 - P)) / d2$, where $Z = 1.96$ for a 95% confidence level, $P = 0.25$ (estimated local H. pylori prevalence among dyspeptic adults), and margin of error $d = 0.05$. This yielded a minimum sample size of $n = 288$. To account for potential sample processing errors or incomplete questionnaires, a total of 304 consenting patients were enrolled. Structured interviewer-administered questionnaires captured demographic variables (age, sex, residence) and clinical history.

Stool Examination Stool examination.

Fresh stool specimens were collected in sterile, leak-proof containers and divided into three aliquots for parallel processing. Active *H. pylori* infection was evaluated using a commercial enzyme-linked immunosorbent assay (ELISA) stool antigen kit (Premier Platinum HpSA, Meridian Bioscience, Cincinnati, OH, USA; diagnostic sensitivity 96.1%, specificity 95.7%) performed strictly according to manufacturer specifications with positive and negative controls.

For parasitological assessment, specimens were examined immediately by direct saline and Lugol's iodine wet mounts, followed by formol-ether sedimentation concentration to optimize detection of protozoan cysts and helminth ova. Slides were examined under ×100 and ×400 light microscopy. Faecal occult blood was screened using a qualitative immunochromatographic rapid test strip (FOB Rapid Test Device, ACON Laboratories, San Diego, CA, USA) to identify mucosal micro-bleeding.

Haematological analysis

Venous blood samples were collected into EDTA tubes at the time of enrolment and analyzed within 2 hours of phlebotomy on a calibrated automated haematology analyser (Sysmex KX-21N, Sysmex Corporation, Kobe, Japan). Recorded haematological parameters included total white blood cell count (WBC), haemoglobin concentration (Hb), haematocrit (HCT), red blood cell count (RBC), and platelet count (PLT). Anaemia was defined per World Health Organization thresholds: haemoglobin < 13.0 g/dL for adult males and < 12.0 g/dL for non-pregnant adult females [5].

Statistical analysis

Data were managed and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Differences in categorical proportions were evaluated using Pearson's chi-square test. Mean differences in haematological parameters between *H. pylori*-positive and negative groups were compared using independent-samples t-tests, with Levene's test applied to check variance equality. To adjust for potential confounding by age and sex and determine the independent association of *H. pylori* infection with anaemia, a multivariable binary logistic regression analysis was conducted, yielding Adjusted Odds Ratios (aOR) with 95% Confidence Intervals (CI). All statistical tests were two-tailed with significance set at $p < 0.05$.

Ethical considerations

The study protocol was approved by the Ethics and Research Committee of Benghazi Medical Centre (Ethics Approval Code: BMC-EC-2024-88). Informed written consent was obtained from all participants prior to enrolment. Anonymized coding ensured participant confidentiality. Patients testing positive for pathogens were referred to attending physicians for standard treatment.

Results

Characteristics of the study population and prevalence of infection

The study cohort comprised 304 participants: 215 females (70.7%) and 89 males (29.3%). Participants ranged across adult age categories, with the highest proportions observed in individuals > 60 years (22.7%) and 31–40 years (21.7%). Overall, *H. pylori* stool antigen was positive in 89 patients, yielding a prevalence of 29.3% (Table 1).

Table 1: Demographic characteristics and H. pylori status of the study population (N = 304)

Characteristic	Category	Frequency (n)	Percentage (%)
Sex	Male	89	29.3
	Female	215	70.7
Age group	≤ 20 years	15	4.9
	21–30 years	50	16.4
	31–40 years	66	21.7
	41–50 years	57	18.8
	51–60 years	47	15.5
	> 60 years	69	22.7
H. pylori stool antigen	Positive	89	29.3
	Negative	215	70.7

Parasitic co-infection and faecal occult blood among infected patients

Among the 89 *H. pylori*-positive individuals, 70 were female (78.7%) and 19 were male (21.3%). Positive cases were concentrated in the 31–40-year age group (44 cases, 49.4%), followed by 41–50 years (24 cases, 27.0%). Stool microscopy revealed *Giardia lamblia* co-infection in 15 patients (16.9%) and *Entamoeba histolytica* in 7 patients (7.9%); no helminths were observed. Faecal occult blood was detected in 56 *H. pylori*-positive patients (62.9%) (Table 2).

Association between *H. pylori* infection and anaemia

Anaemia prevalence was substantially higher among *H. pylori*-infected patients (80/89, 89.9%) compared with uninfected individuals (3/215, 1.4%; $p < 0.001$; **Table 3**). In multivariable binary logistic regression controlling for age and sex, *H. pylori* infection remained strong and independently associated with anaemia (aOR = 210.5, 95% CI: 58.2–761.4, $p < 0.001$). Female sex was also associated with increased odds of anaemia (aOR = 3.2, 95% CI: 1.1–9.4, $p = 0.031$).

Comparison of haematological parameters

Infected patients displayed significant reductions across red cell and platelet parameters alongside elevations in total white cell counts (Table 4). Mean haemoglobin was 9.5 ± 2.0 g/dL in infected patients versus 12.3 ± 1.4 g/dL in uninfected patients ($p < 0.001$). Haematocrit (29.1 ± 5.9 vs 36.3 ± 4.3%,

Table 2: Demographic parameters, parasitic co-infection, and faecal occult blood among *H. pylori*-positive patients (n = 89)

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	19	21.3
	Female	70	78.7
Age group	11–20 years	1	1.1
	21–30 years	15	16.9
	31–40 years	44	49.4
	41–50 years	24	27.0
	51–60 years	2	2.2
	> 60 years	3	3.4
Giardia lamblia	Not detected	74	83.1
	Detected	15	16.9
Entamoeba histolytica	Not detected	82	92.1
	Detected	7	7.9
Faecal occult blood	Negative	33	37.1
	Positive	56	62.9

Table 3: Anaemia distribution by *H. pylori* status

Status	*H. pylori* negative (n = 215)	*H. pylori* positive (n = 89)	p value
Not anaemic	212 (98.6%)	9 (10.1%)	< 0.001**
Anaemic	3 (1.4%)	80 (89.9%)	< 0.001**

p < 0.001) and red cell counts (3.8 ± 0.82 vs 4.3 ± 0.4 × 1012/L, p = 0.009) were similarly lower in the infected cohort. Mean platelet counts were significantly reduced in infected patients (138.8 ± 53.5 vs 282.7 ± 54.4 × 109/L, p < 0.001), whereas total white blood cell counts were nearly double (10.6 ± 3.6 vs 5.9 ± 1.5 × 109/L, p < 0.001).

Table 4: Haematological parameters in *H. pylori*-positive and *H. pylori*-negative patients

Parameter	Positive (n = 89), mean ± SD	Negative (n = 215), mean ± SD	p value
White blood cells (× 10^9/L)	10.6 ± 3.6	5.9 ± 1.5	< 0.001**
Haemoglobin (g/dL)	9.5 ± 2.0	12.3 ± 1.4	< 0.001**
Haematocrit (%)	29.1 ± 5.9	36.3 ± 4.3	< 0.001**
Red blood cells (× 10^{12}/L)	3.8 ± 0.82	4.3 ± 0.4	0.009**
Platelets (× 10^9/L)	138.8 ± 53.5	282.7 ± 54.4	< 0.001**

Independent-samples t-test. ** p < 0.01.

Discussion

This cross-sectional investigation at Benghazi Medical Centre demonstrated a 29.3% stool antigen prevalence *of H. pylori* among adult dyspeptic patients, a 24.7% intestinal protozoan co-infection rate among infected individuals, and a strong independent association between *H. pylori* status and anaemia. These findings align with regional observations across North Africa and the Middle East where gastrointestinal pathogens overlap in clinical settings [3,6,9,10].

The observed *H. pylori* detection rate (29.3%) reflects the diagnostic yield among patients actively seeking specialist evaluation for persistent dyspepsia rather than general community prevalence. Clustering of positive cases in the 31–40-year age bracket (49.4%) is consistent with the natural history of *H. pylori*; wherein bacterial acquisition occurs during early childhood but symptomatic disease and mucosal injury manifest in early to mid-adulthood [3]. The higher female representation among positive cases (78.7%) likely reflects local healthcare utilization patterns and may also contribute to lower baseline iron reserves due to physiological menstrual losses.

The stark difference in anaemia prevalence between H. pylori-positive (89.9%) and negative (1.4%) patients highlights a key clinical finding. Several biological mechanisms account for this marked difference. First, 62.9% of infected patients demonstrated positive faecal occult blood, confirming ongoing micro-bleeding from gastritis or superficial ulceration. Second, the elevated white blood cell counts observed in infected patients (10.6 × 10^9/L vs 5.9 × 10^9/L) signal systemic inflammatory activation. Systemic inflammation drives hepatic hepcidin release, which sequesters macrophage iron reserves and impairs erythropoiesis [5]. This inflammatory response is further driven by persistent gastric bacterial colonization and host immune interaction [2,7].

A notable finding in our dataset was the marked reduction in platelet counts among *H. pylori*-infected patients (138.8 ± 53.5 × 10^9/L vs 282.7 ± 54.4 × 10^9/L, p < 0.001). This mild-to-moderate thrombocytopenia has been increasingly recognized in H. pylori research and is attributed to immune-mediated platelet destruction triggered by molecular mimicry between *H. pylori* CagA/VacA antigens and platelet surface glycoproteins (e.g., GPIIb/IIIa), as well as enhanced platelet clearance by systemic mononuclear phagocytes. Eradication of *H. pylori* has been shown in multiple clinical trials to improve platelet counts in patients with secondary Immune Thrombocytopenic Purpura (ITP) [8].

Co-infection with intestinal protozoa (*Giardia lamblia* in 16.9% and *Entamoeba histolytica* in 7.9%) represents an important concomitant finding [6,9,10,11]. Giardia causes villous flattening in the proximal duodenum, impairing active iron absorption, while *E. histolytica* induces mucosal erosion in the colon [11]. However, because the absolute numbers of co-infected individuals were small (n = 15 for *G. lamblia* and n = 7 for *E. histolytica*), statistical comparisons of haematological parameters between *H. pylori* mono-infected and co-infected subgroups were underpowered. Consequently, while concurrent parasitic infection represents a plausible additive factor, this study design describes observational associations rather than a proven synergistic mechanism. Larger multi-centre prospective studies are needed to evaluate subgroup differences directly.

Limitations

Several study limitations must be noted. First, the cross-sectional design captures exposure and outcome simultaneously, precluding definitive conclusions regarding temporal or causal relationships. Second, enrollment from a single tertiary referral facility limits generalizability to asymptomatic community populations. Third, diagnostic testing relied on stool antigen ELISA and light microscopy without endoscopic confirmation, biopsy histopathology, or urea breath testing. Fourth, specific iron parameters (serum ferritin, total iron-binding capacity, transferrin saturation) and detailed red cell indices (MCV, MCH, RDW) were not included in the standard panel, preventing formal differentiation between iron deficiency anaemia, anaemia of chronic disease, or mixed etiologies. Finally, unmeasured potential confounders such as dietary iron intake, dietary habits, socioeconomic background, and non-steroidal anti-inflammatory drug (NSAID) use could not be adjusted for in

statistical models.

Conclusions and Recommendations

Among adult dyspeptic patients at Benghazi Medical Centre, H. pylori infection was strongly associated with anaemia, reduced red blood cell parameters, thrombocytopenia, and positive faecal occult blood. Intestinal protozoan co-infections were present in a noteworthy minority of infected patients. Based on these observations, the following recommendations are made:

1. Integrated Screening: Clinical management of chronic dyspepsia presenting with low haemoglobin should incorporate concurrent stool antigen testing for H. pylori and microscopic parasite screening alongside standard haematological evaluation.
2. Comprehensive Diagnostic Workup: Where available, complete iron profile testing (serum ferritin and iron markers) should be performed to guide clinical management.
3. Preventive Public Health: Community health education promoting clean water access and sanitation remains essential to break faecal-oral transmission of both bacterial and parasitic gut pathogens [1,3].
4. Future Research: Prospective longitudinal studies with full iron panels and therapeutic follow-up are warranted to quantify haematological recovery following pathogen eradication.

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