ORIGINAL ARTICLE

Frequency of Vitamin B12 Deficiency and Iron Deficiency Anemia in Chronic Proton Pump Inhibitor Users

Haleema Gul[1*], Tariq Hussain[1], Shabana Mehr[1], Abdullah[1], Yasir Saadat[1] and Shoukat Ali[1]

[1]*PAEC General Hospital, Islamabad, Pakistan*

Abstract

Background: Proton pump inhibitors (PPIs) are widely prescribed for acid-related gastrointestinal disorders, but long-term use may impair absorption of essential micronutrients, including vitamin B12 and iron, leading to deficiency and anemia.

Objective: To determine the frequency of vitamin B12 deficiency and iron deficiency anemia in chronic PPI users.

Methods: A cross-sectional study was conducted at the Department of General Medicine at Pakistan Atomic Energy Commission Hospital (PAEC), Islamabad, Pakistan, from May 2024 to October 2024. In this study, 200 adult chronic PPI users were enrolled from outpatient clinics and general medicine wards. Vitamin B12 deficiency refers to serum B12 levels less than 200 pg/mL, and iron deficiency anemia refers to low hemoglobin (sex-specific) and low serum ferritin (less than 15 ng/mL) or transferrin saturation (less than 20%).

Results: The average age of the participants was 48.6 ± 12.4 years, and the proportion of females was 54%. Normal B12 levels were found in half of the patients, whereas borderline and <200 B12 levels were found in 29% of patients. The frequency of iron deficiency anemia was 22%. The concomitant frequency of B12 deficiency and iron deficiency anemia was 9%. The use of PPI over a period of time (>24 months) was significantly associated with increased risks of vitamin B12 deficiency (aOR=3.42, 95% CI: 1.67-7.00, p<0.001) and iron deficiency anemia (aOR=2.95, 95% CI: 1.31-6.62, p=0.009).

Conclusion: The chronic use of PPIs is associated with a significant risk of vitamin B12 and iron deficiency, especially in patients on long-term therapy and those with low intake of animal protein.

Keywords: *Iron deficiency anemia, proton pump inhibitor, vitamin B12, prevalence.*

INTRODUCTION

Long-term use of proton-pump inhibitors (PPIs) is common worldwide. PPIs, including omeprazole, pantoprazole, lansoprazole, rabeprazole, and esomeprazole, are highly effective acid-suppressing agents and are prescribed for gastroesophageal reflux disease (GERD), peptic ulcer disease, H. pylori eradication regimens, Zollinger-Ellison syndrome, and as prophylaxis in selected high-risk patients [1, 2]. Although short-term use is generally safe, concerns have mounted about adverse outcomes with chronic therapy, including nutrient malabsorption and consequent micronutrient deficiencies. These nutritional risks are biologically plausible because gastric acid has an important role in the liberation, solubilization, and subsequent intestinal absorption of several nutrients, most notably iron and vitamin B12 [3, 4].

Mechanisms linking PPIs to vitamin B12 and iron deficiency are well described [5]. Dietary vitamin B12in food is protein-bound and requires gastric acid and pepsin to release it from food proteins; the released cobalamin then binds intrinsic factor in the stomach and is absorbed in the terminal ileum [6]. By raising gastric pH, PPIs reduce proteolytic release of food-bound B12 and may therefore reduce its bioavailability over time. Iron, particularly non-heme iron, requires solubilization in an acidic environment to convert ferric (Fe3+) to the more absorbable ferrous (Fe2+) form; hypochlorhydria secondary to PPI therapy can therefore impair iron solubilization and reduce intestinal uptake. Additional indirect mechanisms may include PPI-driven changes in the gastric microbiota, altered secretion of gastric hormones such as gastrin, or clinical situations that coexist with PPI use, such as older age, chronic disease, or concomitant medications that independently affect nutrient status [4, 7].

Large systematic reviews and meta-analyses, as well as cohort studies, showed mixed but concerning evidence. A pooled analysis of studies assessing vitamin B12 status found a modestly increased odds of B12 deficiency among chronic PPI users compared with non-users (pooled OR ≈ 1.4), although heterogeneity between studies and differences in how B12 deficiency was defined limited the strength of the conclusion. Several individual studies reported no association, while others found a higher prevalence in long-term users. These inconsistencies likely reflect variable exposure definitions, differing B12 assays, and confounders such as age, metformin use, and atrophic gastritis [8-10].

Evidence is somewhat stronger for an association between chronic PPI use and iron deficiency/anemia. A recent systematic review and meta-analysis reported that PPI users had a higher risk of iron deficiency

**Corresponding author: Haleema Gul, PAEC General Hospital, Islamabad, Pakistan, Email: Haleemaikram2k18@gmail.com*
Received: December 12, 2025; Revised: February 28, 2026; Accepted: March 27, 2026
DOI: https://doi.org/10.37184/lnjpc.2707-3521.8.48

  ISSN: 2708-9134 (Online) Liaquat National Journal of Primary Care 2026; 8(3): 296-302

anemia, and several cohort and transplant-recipient studies have described higher odds of iron deficiency in persistent PPI users, with risk appearing to rise with longer duration and more potent acid suppression [11, 12]. Still, iron deficiency is multifactorial (diet, blood loss, malabsorption syndromes, surgical history); thus, PPI exposure should be considered one of several potential contributors rather than a sole cause in most patients [13-15].

Given the biological plausibility, the high and often prolonged use of PPIs, and the potential for reversible but clinically important consequences, quantifying the frequency of B12 deficiency and iron deficiency anemia among chronic PPI users is important. Precise local data are also needed because prevalence will vary by population age-structure, dietary patterns, comorbidities, and prescribing practices. Robust estimates will inform screening recommendations, guide counseling about dietary sources and supplementation, and help clinicians weigh long-term risks against benefits when continuing PPI therapy. The present study aimed to determine the frequency of vitamin B12 deficiency and iron deficiency anemia in chronic PPI users.

METHODS

This study employed a cross-sectional design and was conducted in the Department of General Medicine at the Pakistan Atomic Energy Commission Hospital (PAEC), Islamabad, Pakistan. The study was performed from May 2024 to October 2024. Patients attending the outpatient clinics and those admitted to the general medicine wards who met the eligibility criteria were screened and recruited. Ethical approval was obtained from the Institutional Ethical Committee (IEC) of Postgraduate Institute of Hematology and Immunology (PGHI) (Approval no.: PGHI-IRB (DMe)-RCD_06-087, dated: 17th April 2024).

The sample size was calculated using the WHO sample size calculator. An anticipated prevalence of 55.1% for vitamin B12 deficiency among chronic proton pump inhibitor users was assumed based on previously reported ranges in the literature [9]; a 95% confidence level and a 5% absolute precision were used. After these considerations, the final sample size required was 200 patients.

Eligible participants were enrolled by non-probability consecutive sampling. Adults aged 18 years or older who had been taking any oral proton pump inhibitor continuously for at least six months before enrollment were eligible for inclusion. Exclusion criteria included a documented diagnosis of vitamin B12 deficiency or iron deficiency anemia before initiating proton pump inhibitor therapy, use of vitamin B12 or iron supplements within the preceding three months, prior gastric surgery, known malabsorption syndromes, active gastrointestinal bleeding or known chronic blood-loss disorders, chronic inflammatory diseases or malignancy expected to influence iron indices, end-stage renal disease, pregnancy, and current use of other medications known to affect B12 or iron status substantially. Concomitant medications that were common in the population were recorded and later adjusted for in the analysis rather than used as exclusion criteria. Patients willing to participate provided written informed consent.

A structured data collection form was used to record patients' information. After eligibility screening and written informed consent, trained investigators interviewed patients to obtain demographic data (age, sex), clinical history (indication for proton pump inhibitor use, specific proton pump inhibitor agent, daily dose, and duration of use in months), dietary history focusing on animal-source food intake, menstrual history for women of reproductive age, history of gastrointestinal symptoms, relevant past medical history (diabetes, autoimmune disease), and current medication list (including metformin, antiplatelets/anticoagulants, non-steroidal anti-inflammatory drugs). The majority of patients were receiving omeprazole at a daily dose of 20-40 mg, while smaller proportions were using other PPIs such as pantoprazole (40 mg daily) and esomeprazole (20-40 mg daily) as part of routine clinical care. A brief focused physical examination was performed to document pallor, jaundice, glossitis, neurological findings, and anthropometric measures.

Venous blood (7-10 mL) was collected aseptically by trained phlebotomists. CBC samples were collected in EDTA microflasks and analyzed on the same day using an automated hematology analyzer. Plain tubes were used to collect serum samples, which were clotted, centrifuged, and tested immediately or stored at 2-8°C until analysis was completed within 48 hours. A chemiluminescent immunoassay was used to measure vitamin B12. Hemoglobin, red cell indexes, serum ferritin, and (where available) serum iron and TIBC were used to assess iron status and determine transferrin saturation. Laboratory tests were performed in accordance with standard quality control protocols.

Vitamin B12 deficiency was defined as serum vitamin B12 below 200 pg/mL (148 pmol/L), the laboratory reference interval as indicated by the manufacturer of the vitamin B12 assays. Normal ranges were 200-300 pg/mL (148-221 pmol/L); these were considered borderline in the clinical setting, but only those below 200 pg/mL were deemed deficient in this study. A chemiluminescent immunoassay was used to measure serum vitamin B12 as indicated by its manufacturer. The assay supplier provided the laboratory reference cut-off for deficiency (<200 pg/mL). This cut-off has not been locally confirmed by known cases of functional B12 deficiency in our population; hence, it should be interpreted as indicating a possibility of deficiency rather than a definite deficiency.

Iron deficiency anemia was classified according to WHO criteria, combining hemoglobin with sex-specific cut-offs

and low serum ferritin (or low transferrin saturation when ferritin was equivocal), while considering inflammatory conditions [16]. Anemia was defined as a hemoglobin level of less than 13.0 g/dl in men and less than 12.0 g/dl in women. Serum ferritin below 15 ng/mL (ug/L), defined as iron deficiency, was used, with a higher cut-off of below 30 ng/mL in cases of possible inflammation. In equivocal ferritin values, transferrin saturation of less than 20% (calculated as the ratio of serum iron to TIBC) was used to confirm iron deficiency. Patients who met criteria for both anemia and iron deficiency were rolled into the iron-deficiency anemia category. All participants with abnormal outcomes were counseled and referred to ensure accurate clinical care. Conversely, vitamin B12 status was determined solely by plasma vitamin B12 levels, which do not always reflect functional or tissue deficiency. Thus, B12 and iron deficiency cannot be equated as similarly defined deficiencies.

SPSS version 26 was used to analyze the data. Mean ± SD were used to summarize continuous variables, and frequencies and percentages to summarize categorical variables. The prevalence of vitamin B12 deficiency and iron deficiency anemia was estimated using 95% confidence intervals. The chi-square test of categorical data was used to compare patients' categorical features among those with and without B12 deficiency. Binary logistic regression was used to determine the association between patients' features and B12 deficiency and iron deficiency anemia. Both crude and adjusted odds ratios were computed, along with their 95% confidence intervals. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The mean age of the 200 respondents was 48.6 ± 12.4 years, and the study group included 54% females. The study participants used PPI for a mean duration of 19.2 ± 9.8 months. More than one-third of patients had been taking them within 6-12 months (37%), 41% between 12-24 months, and 22% more than 24 months. The most used agent was omeprazole (49%), followed by pantoprazole (32%); esomeprazole and other PPIs accounted for 13% and 6%, respectively. The participants were also reviewed on the daily doses of proton pump inhibitors. Animal-source food consumption was reported to be regular among 61% of participants, and diabetes and metformin use were reported among 21% and 19%, respectively (**Table 1**).

Table 1: Baseline characteristics of study Participants (n=200).

Characteristic	Statistics
Age (years), mean ± SD	48.6 ± 12.4
Gender, n(%)	
Male	92 (46.0)
Female	108 (54.0)
Mean Duration of PPI Use (months), mean ± SD	19.2 ± 9.8
Duration of PPI Use, n(%)	
6-12 months	74 (37.0)
>12-24 months	82 (41.0)
>24 months	44 (22.0)
PPI Type, n(%)	
Omeprazole	98 (49.0)
Pantoprazole	64 (32.0)
Esomeprazole	26 (13.0)
Others	12 (6.0)
Dietary Animal-Source Food (≥3/week), n(%)	122 (61.0)
Diabetes Mellitus, n(%)	42 (21.0)
Metformin Use, n(%)	38 (19.0)

The participants were also grouped by the approximate intensity of acid suppression, based on PPI type and daily dosage. Iron deficiency anemia was found in 22 of 84 participants (26.2%) taking higher-dose acid-suppressive regimens, compared with 22 of 116 participants (19.0%) taking standard-dose regimens. The difference, however, was not significantly different (p=0.210) (**Table 2**).

Table 2: Comparison of iron deficiency anemia according to intensity of PPI-induced acid suppression (n=200).

Acid Suppression Category	Iron Deficiency Anemia		p-value
	Present n(%)	Absent n(%)	
Higher acid suppression (omeprazole 40 mg/day or equivalent)	22 (26.2)	62 (73.8)	0.210
Standard dose (omeprazole 20 mg/day or equivalent)	22 (19.0)	94 (81.0)	

A total of 58 participants (29%) had plasma vitamin B12 concentrations below the laboratory reference threshold (<200 pg/mL), indicating possible deficiency or high risk of deficiency. Anemia caused by iron deficiency was found in 22.0%. It is noteworthy that both vitamin B12 deficiency and iron deficiency anemia were present among 9.0% of participants (95% CI: 5.0-13.0) (**Table 3**).

Table 3: Prevalence of vitamin B12 deficiency and iron deficiency anemia (n=200).

Variables	n(%)	95% CI
Serum B12		
Possible B12 Deficiency (<200)	58 (29.0)	22.6-35.4
Borderline (200-300)	42 (21.0)	12.0-22.0
Normal (>300)	100 (50.0)	47.0-61.0
Iron Deficiency Anemia	44 (22)	16.2-27.8
Both Vitamin B12 Deficiency + Iron Deficiency Anemia	18 (9.0)	5.0 - 13.0

CI represents the 95% confidence interval for the proportion of patients with each outcome.

The average age of the deficient patients was significantly higher than that of the non-deficient patients (p=0.048). There was a disproportionate impact on females, with 65.5% of the deficient persons being women, compared with 49.3% in the non-deficient group (p=0.041). Prolonged PPI use and B12 deficiency showed a close relationship: 44.8% of the study participants with B12

deficiency had taken PPI for more than 24 months, compared with 12.7% in controls (p<0.001). The use of Metformin was increased in deficient patients (27.6% *vs.* 15.5%), but was not statistically significant (p=0.067). The diets also played an important role, as intake of low-animal-source foods was noted in 51.7% of the deficient and 33.8% of the non-deficient participants (p=0.023) (**Table 4**).

Table 4: Comparison of participants with and without vitamin B12 deficiency (n=200).

Variables	B12 Deficiency	No Deficiency	p-value
Age in years (mean ± SD)	51.2 ± 11.8	47.4 ± 12.6	*0.048
Female sex, n (%)	38 (65.5)	70 (49.3)	*0.041
Duration of PPI >24 months, n (%)	26 (44.8)	18 (12.7)	*<0.001
Metformin use n (%)	16 (27.6)	22 (15.5)	0.067
Low animal-source diet, n (%)	30 (51.7)	48 (33.8)	*0.023

*Significant at p<0.05

In multivariable analysis, prolonged PPI use beyond 24 months was a strong predictor of both deficiencies, with affected participants being over three times more likely to develop vitamin B12 deficiency (p=0.001) and nearly three times more likely to develop iron deficiency anemia (p=0.009) than those with shorter use. Female sex was independently associated with a higher risk, increasing the odds of B12 deficiency by 78% (p=0.045) and more than doubling the risk of iron deficiency anemia (p=0.015). Low intake of animal-source foods was also significantly associated with B12 deficiency (p=0.013). Additionally, heavy menstrual history was a notable risk factor for iron deficiency anemia, tripling the odds (p=0.008). The concomitant use of Metformin was also assessed, as Metformin has been reported to affect vitamin B12 absorption and status, which might have confounded the effect of PPI use on B12 deficiency. This possible confounding factor could be adjusted by including Metformin as a covariate in multivariate analyses (**Table 5**).

Table 5: Univariate and multivariable logistic regression analysis for association of vitamin B12 deficiency and iron deficiency anemia (n=200).

Variables	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Vitamin B12 Deficiency				
Duration of PPI >24 months	5.63 (2.83-11.17)	*<0.001	3.42 (1.67-7.00)	*0.001
Female sex	1.95 (1.05-3.62)	*0.034	1.78 (1.01-3.15)	*0.045
Low animal-source diet	2.05 (1.14-3.70)	*0.017	2.21 (1.18-4.12)	*0.013
Metformin use	2.07 (1.01-4.25)	*0.047	1.56 (0.77-3.12)	0.212
Iron Deficiency Anemia				
Duration of PPI >24 months	3.68 (1.78-7.62)	*<0.001	2.95 (1.31-6.62)	*0.009
Female sex	2.95 (1.46-5.94)	*0.002	2.42 (1.19-4.94)	*0.015
Menstrual history (heavy)	3.84 (1.72-8.56)	*0.001	3.10 (1.34-7.15)	*0.008

CI: Confidence interval, OR: Odds ratio, *Significant at p<0.05

DISCUSSION

In this cross-sectional sample of 200 chronic PPI users, we found a high frequency of micronutrient problems: vitamin B12 deficiency in 29.0% and iron deficiency anemia in 22.0%, with both deficiencies coexisting in 9.0%. These prevalences are substantial and align with a body of recent literature reporting an increased risk of vitamin and iron deficits among long-term acid suppressor users, while also reflecting the heterogeneity of previous studies in terms of magnitude and methods [8, 13, 17].

The present study findings of B12 are higher than many single-center reports, but are consistent with analyses that link prolonged and more potent acid suppression to impaired B12 status. A 2023 meta-analysis found a modestly increased odds of B12 deficiency in PPI users (pooled OR ≈1.4), though the authors highlighted important between-study heterogeneity and differences in how deficiency was defined [8]. Similarly, studies of patients with severe, long-standing acid suppression have reported notable rates of functional B12 deficiency, particularly when objective functional biomarkers (MMA, homocysteine, holo-TC) are used. These comparisons suggest our relatively high prevalence may reflect true exposure-related risk and differences in population diet and comorbidity [4, 5, 18].

The observed prevalence of iron deficiency anemia (22%) also echoes recent systematic reviews and cohort data that implicate PPI therapy in impaired iron handling. Several studies on PPI use and iron deficiency/anemia reported elevated risk estimates for long-term PPI users, and several cohort analyses (including cohorts of transplant recipients and other high-risk groups) found stronger associations with higher PPI dose and with more potent agents. Our finding that PPI duration >24 months strongly predicted both B12 deficiency and iron deficiency anemia is directly concordant with dose/duration-response signals reported in those cohorts [14, 17, 19].

Mechanistically, our results are biologically plausible. Gastric acid facilitates the release of protein-bound vitamin B12 in food and converts dietary ferric iron (Fe^{3+}) to the more absorbable ferrous form (Fe^{2+}). Experimental and translational work has demonstrated that acid suppression can impair these chemical steps and reduce iron uptake, supporting clinical associations between PPIs and iron deficiency. This mechanistic literature strengthens the likelihood that at least part of

the observed associations is causal rather than purely confounded [5, 7, 20-22].

Not all studies, however, find strong or consistent associations. Several well-conducted observational studies and some smaller clinical series reported no statistically significant link between routine PPI use and lower serum B12 or homocysteine, and meta-analyses emphasize heterogeneity in exposure definitions, laboratory assays, and confounding (age, metformin use, dietary intake, atrophic gastritis). For example, a number of 2022 cohort studies did not find an association when only single serum B12 values were used or when short-term users were included, suggesting that confounding by indication, differences in biomarker selection (total B12 *vs* functional markers), and population diet can materially affect results. These inconsistencies highlight why our findings of higher prevalence should be interpreted alongside assay methods and local population characteristics [18, 23].

Certain subgroups appear to be at higher risk across multiple reports, and this is mirrored in our data. Female sex, long duration of therapy (>24 months), low intake of animal-source foods, and (to a lesser extent) concurrent metformin use was associated with deficiency in our sample, findings that are echoed in other analyses which identify older age, female sex, heavier menstrual losses, and polypharmacy (metformin, antiplatelet or anticoagulant use) as modifiers of risk. These concordant subgroup effects strengthen clinical plausibility and point to whom screening efforts might be prioritized [9, 24, 25].

Strengths of our study include a clear exposure definition, concurrent measurement of B12 and iron indices, and multivariable modeling to adjust for major confounders. These limitations that moderate interpretation are common to much of the existing literature: cross-sectional design, use of single-time-point serum assays, potential residual confounding variables such as unmeasured atrophic gastritis, occult blood loss, and the size of our sample and single-center recruitment are limiting the generalizability. Through meta-analysis, variability of biomarker choice and exposure threshold has been repeatedly observed to decrease comparative ability across studies; thus, longitudinal cohorts with standardized biomarker panels would yield better causation.

There are a number of limitations in this study. Its cross-sectional nature makes it impossible to draw causal conclusions, and recruitment is only through a single center, which might not be generalizable. Functional markers of vitamin B12, such as methylmalonic acid or holo-transcobalamin, which might have better reflected vitamin B12 status, were not used, but just single-time-point serum measurements. Possible residual confounding factors, including undetermined atrophic gastritis, occult gastrointestinal bleeding, and detailed dietary intake, might affect results. The study sample size, which is sufficient in estimating the overall prevalence, may limit power in detecting dosage-dependent effects. Even though the dose of PPI taken per day was captured in the course of interviewing patients, not all of them were fully documented, and thus could not be analyzed. Thus, the dose effect on acid inhibition and the following vitamin B12 or iron deficiency could not be measured. Subsequent research would be required to record full dosing data to investigate dose-effective changes. The observed micronutrient deficiencies could be affected by differences in PPI potency and daily dose. Although subgroup analyses by acid suppression were conducted, the study did not have enough power to understand the dose-response effects conclusively. The current paper presents biochemical findings that contribute to vitamin B12 and iron deficiencies in the form of vitamin B12 levels in serum, hemoglobin, and ferritin levels. No systematic evaluation of clinical manifestations of deficiency was done, and functional indicators like methylmalonic acid or homocysteine have not been measured. The functional and clinical assessment should be incorporated into future studies to prove the deficiency and get a more precise understanding of its effects on patient outcomes. Subclinical or marginal malnutrition might have contributed to baseline micronutrient status and vulnerability to depletion due to PPI. To explain the role of nutritional intake in micronutrient deficiencies, future research should use objective measures of protein status. Participants were not specifically excluded, even though patients with gastrointestinal disorders were excluded, though not the participants with marginal or low dietary intake. They did not measure detailed quantitative dietary intake and baseline body stores of vitamin B12 and iron, which could affect the rate of depletion during the chronic use of PPI. Because only single-time-point measurements were obtained, potential variability in vitamin B12 and iron indices could not be accounted for. This limitation should be considered when interpreting prevalence estimates and associations with PPI use.

Clinical implications and recommendations: due to the consistent mechanistic explanation and numerous reports (including our own) depicting significant prevalences of deficiency amid the period of long-term PPI usage, clinicians must periodically re-examine the rationale underpinning the ongoing PPI treatment and think about specific screening in more susceptible patients (duration over 1224 months, women with heavy menses, poor dietary consumption of animal-source foods and those on Metformin). In case of a suspected deficiency, appropriate biomarkers (including functional markers as indicated) should be determined and corrected promptly by either nutritional or pharmacologic means. Lastly, prospective designs, standardized deficiency (including functional measures of MMA/holo-TC), and dose/potency effects in diverse populations should be the focus of future research.

CONCLUSION

Chronic proton pump inhibitor (PPI) use is largely linked with a high rate of vitamin B12 deficiency (29%) and

iron deficiency anemia (22%), and almost one out of ten patients has both vitamin B12 deficiency and iron deficiency anemia. These results support the biological feasibility that chronic acid repression can attenuate nutrient assimilation and emphasize the necessity to oversee susceptible groups of people, especially women, chronic users, and people with unhealthy eating behaviors. Given the widespread and often prolonged prescription of PPIs, our results emphasize the need for judicious prescribing, regular re-evaluation of therapy duration, and targeted screening for micronutrient deficiencies. Early detection and timely intervention can help prevent potentially serious hematological and neurological consequences, ensuring safe and rational use of PPIs in clinical practice.

ETHICS APPROVAL

Ethical approval was obtained from the Institutional Ethical Committee (IEC) of Postgraduate Institute of Hematology and Immunology (PGHI), approval no: PGHI-IRB (DMe)-RCD_06-087, dated: 17th April 2024. All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Written consent was obtained from the participants.

AVAILABILITY OF DATA

The data can be shared after obtaining consent from all the concerned authors, depending on the need for data.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

AUTHORS' CONTRIBUTION

Haleema Gul	: Conception and design of study, manuscript writing, and final editing.
Tariq Hussain	: Study supervision, critical review, and intellectual input in manuscript editing.
Shabana Mehr	: Data collection, literature search, and contribution to manuscript writing.
Abdullah	: Data collection, data entry, and assistance in manuscript drafting.
Yasir Saadat	: Data analysis, interpretation of results, and review of manuscript.
Shoukat Ali	: Crtical review and revision of manuscript and work supervision.

All authors read and approved the final version of the manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of the present work, the authors limitedly used ChatGPT (GPT-4, OpenAI) to get language suggestions and do minor proofreading in some parts of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

REFERENCES

1. Mansour MB, Ahmedana SE, Basheer AM. The wise long-term use of proton pump inhibitors (PPIs) among adults in hospitals, health centers, and clinics. World Fam Med 2024; 22(4): 25-38. DOI: https://doi.org/10.5742/MEWFM.2024.95257634
2. Moustafa HAM, Al Meslamani AZ, Ahmed HMME, Ahmed SAF, Sallam NES, Alshehri GH, *et al.* Knowledge, attitudes, and practices of community pharmacists regarding proton pump inhibitor (PPI) use: a cross-sectional study. Healthcare (Basel) 2025; 13(13): 1588. DOI: https://doi.org/10.3390/healthcare13131588
3. Losurdo G, Caccavo NLB, Indellicati G, Celiberto F, Ierardi E, Barone M, *et al.* Effect of long-term proton pump inhibitor use on blood vitamins and minerals: a primary care setting study. J Clin Med 2023; 12(8): 2910. DOI: https://doi.org/10.3390/jcm12082910
4. Ito T, Ramos-Alvarez I, Jensen RT. Long-term proton pump inhibitor-acid suppressive treatment can cause vitamin B12 deficiency in Zollinger-Ellison syndrome (ZES) patients. Int J Mol Sci 2024; 25(13): 7286. DOI: https://doi.org/10.3390/ijms25137286
5. Sanam S. Pharmacokinetic drug interactions of multivitamins and proton-pump inhibitors. 2024; University of Dhaka. Available from: http://reposit.library.du.ac.bd: 8080/xmlui/xmlui/handle/123456789/3465 [Accessed 2025]
6. Moravcová M, Siatka T, Krčmová LK, Matoušová K, Mladěnka P; OEMONOM. Biological properties of vitamin B12. Nutr Res Rev 2025; 38(1): 338-70. DOI: https://doi.org/10.1017/S0954422424000210
7. Hamano H, Niimura T, Horinouchi Y, Zamami Y, Takechi K, Goda M, *et al.* Proton pump inhibitors block iron absorption through direct regulation of hepcidin *via* the aryl hydrocarbon receptor-mediated pathway. Toxicol Lett 2020; 318: 86-91. DOI: https://doi.org/10.1016/j.toxlet.2019.10.016
8. Choudhury A, Jena A, Jearth V, Dutta AK, Makharia G, Dutta U, *et al.* Vitamin B12 deficiency and use of proton pump inhibitors: a systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol 2023; 17(5): 479-87. DOI: https://doi.org/10.1080/17474124.2023.2204229
9. Mumtaz H, Ghafoor B, Saghir H, Tariq M, Dahar K, Ali SH, *et al.* Association of vitamin B12 deficiency with long-term PPIs use: a cohort study. Ann Med Surg (Lond) 2022; 82: 104762. DOI: https://doi.org/10.1016/j.amsu.2022.104762
10. Dharmarajan TS, Norkus EP. Does long-term PPI use result in vitamin B12 deficiency in elderly individuals? Nat Clin Pract Gastroenterol Hepatol 2008; 5(11): 604-5. DOI: https://doi.org/10.1038/ncpgasthep1263
11. Wu YT, Lu YT, Chu CY, Chao HJ, Kuo LN, Cheng KJ, *et al.* Is use of a long-term proton pump inhibitor or histamine-2 receptor antagonist a risk factor for iron-deficiency anaemia in Taiwan? A neglected clinical drug-drug interaction. Fam Pract 2025; 42(2): cmad090. DOI: https://doi.org/10.1093/fampra/cmad090
12. ul Ain Q, Saeed E, Noreen N, Asgher N, Farooq U. Chronic use of proton pump inhibitor leading to iron deficiency anemia in a middle-aged woman. J Bahria Univ Med Dent Coll 2025; 15(3): 265-7. DOI: https://doi.org/10.51985/JBUMDC2025537

13. Ali MD. Proton pump inhibitors' use and risk of iron deficiency anaemia: a systematic review and meta-analysis. Curr Rev Clin Exp Pharmacol 2023; 18(2): 158-66.
DOI: https://doi.org/10.2174/2772432817666220307121220

14. Douwes RM, Vinke JSJ, Gomes-Neto AW, Ayerdem G, van Hassel G, Berger SP, *et al.* Type of proton-pump inhibitor and risk of iron deficiency in kidney transplant recipients: results from the TransplantLines Biobank and Cohort Study. Transpl Int 2021; 34(11): 2305-316.
DOI: https://doi.org/10.1111/tri.14110

15. Koyyada A. Long-term use of proton pump inhibitors as a risk factor for various adverse manifestations. Therapie 2021; 76(1): 13-21.
DOI: https://doi.org/10.1016/j.therap.2020.06.019

16. Neef V, Schmitt E, Bader P, Zierfuß F, Hintereder G, Steinbicker AU, *et al.* The reticulocyte hemoglobin equivalent as a screening marker for iron deficiency and iron deficiency anemia in children. J Clin Med 2021; 10(16): 3506.
DOI: https://doi.org/10.3390/jcm10163506

17. Chinzon D, Domingues G, Tosetto N, Perrotti M. Safety of long-term proton pump inhibitors: facts and myths. Arq Gastroenterol 2022; 59(2): 219-25.
DOI: https://doi.org/10.1590/S0004-2803.202202000-40

18. Swarnakari KM, Bai M, Manoharan MP, Raja R, Jamil A, Csendes D, *et al.* The effects of proton pump inhibitors in acid hypersecretion-induced vitamin B12 deficiency: a systematic review. Cureus 2022; 14(11): e31672.
DOI: https://doi.org/10.7759/cureus.31672

19. Prabhu K, Warricker F, Almilaji O, Williams E, Snook J. Role of prescribed medication in the development of iron deficiency anaemia in adults: a case-control study. BMJ Open Gastroenterol 2024; 11(1): e001305.
DOI: https://doi.org/10.1136/bmjgast-2023-001305

20. Rahamn MR, Ahmed QM, Rahim MA, Sami CA, Ahmad HI, Hasan MN. Correlation of long-term proton pump inhibitors (PPI) use with iron and vitamin B12 deficiency anaemia. Bangladesh Med J 2021; 50(1): 27-32.
DOI: https://doi.org/10.3329/bmj.v50i1.58250

21. Auerbach M, DeLoughery TG, Tirnauer JS. Iron deficiency in adults: a review. JAMA 2025; 333(20): 1813-23.
DOI: https://doi.org/10.1001/jama.2025.0452

22. Hailstone E, Falkos S, Vidal R, Jones KA, Gaillard PR, Fan S, *et al.* Impact of acid suppression therapy on iron supplementation in the pediatric intensive care unit. J Pediatr Pharmacol Ther 2021; 26(4): 366-71.
DOI: https://doi.org/10.5863/1551-6776-26.4.366

23. Lerman TT, Cohen E, Sochat T, Goldberg E, Goldberg I, Krause I. Proton pump inhibitor use and its effect on vitamin B12 and homocysteine levels among men and women: a large cross-sectional study. Am J Med Sci 2022; 364(6): 746-51.
DOI: https://doi.org/10.1016/j.amjms.2022.07.006

24. Mir AA, Wani ZA, Bhat AR, Fahelelbom KM, Kumar A, Ahmed S. Widening spectrum of adverse effects caused by long-term use of proton pump inhibitors: a comprehensive review of literature. LabMed Discovery 2024; 100027.
DOI: https://doi.org/10.1016/j.lmd.2024.100027

25. Obeid R, Andrès E, Češka R, Hooshmand B, Guéant-Rodriguez RM, Prada GI, *et al.* Diagnosis, treatment and long-term management of vitamin B12 deficiency in adults: a Delphi expert consensus. J Clin Med 2024; 13(8): 2176.
DOI: https://doi.org/10.3390/jcm13082176