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Original Article

Effect of Proton Pump Inhibitor Use on Serum Magnesium, Iron, and Vitamin B12 Levels: A Duration-Dependent Analysis

Corresponding Author:
Dr. Niranjan Saran

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Niranjan Saran[1], Shilpa Gupta[2]

[1]Associate Professor, Department of Pharmacology, MGM Medical College & LSK Hospital, Kishanganj, Bihar, India

[2]Assistant Professor, Department of Biochemistry, Shri Gorakshnath Medical College Hospital and Research Centre, Uttar Pradesh, India.

ABSTRACT

Background: Proton pump inhibitors (PPIs) are among the most widely prescribed drugs worldwide and are frequently continued for years beyond their approved indications. Gastric acid suppression impairs the intestinal absorption of magnesium, non-haem iron and food-bound vitamin B12, but the extent to which these effects accumulate with duration of therapy is incompletely characterised in Indian patients.

Objectives: To compare serum magnesium, iron indices and vitamin B12 across three strata of PPI exposure (<12, 12–36 and >36 months) and to quantify the relationship between duration of PPI use and each biochemical parameter.

Methods: A hospital-based cross-sectional study was conducted at MGM Medical College & LSK Hospital, Kishanganj, Bihar, from August 2025 to June 2026. Sixty adults on continuous PPI therapy were enrolled consecutively, 20 in each duration group. Serum magnesium, iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin, vitamin B12, haemoglobin and creatinine were measured. Groups were compared by one-way ANOVA with Tukey post-hoc testing and chi-square tests; the duration–outcome relationship was examined by Pearson correlation and multivariable linear and logistic regression.

Results: Mean age was 50.6 ± 6.9 years; 51.7% were women. The three groups were comparable for age, sex, body mass index, indication, PPI molecule, dose and adherence. Serum magnesium (2.02 ± 0.16 vs 1.90 ± 0.13 vs 1.76 ± 0.15 mg/dL), serum iron (100.0 ± 19.1 vs 90.3 ± 12.0 vs 63.3 ± 11.8 µg/dL), ferritin (80.6 ± 16.3 vs 61.0 ± 18.4 vs 38.7 ± 20.8 ng/mL), transferrin saturation (31.0 ± 5.6 vs 26.9 ± 3.3 vs 17.4 ± 3.9%) and vitamin B12 (392.5 ± 56.4 vs 310.6 ± 61.9 vs 219.0 ± 73.7 pg/mL) declined progressively across the <12, 12–36 and >36-month groups (all $p < 0.001$). Hypomagnesaemia (0%, 5%, 35%; $p = 0.002$), iron deficiency (0%, 0%, 80%; $p < 0.001$) and vitamin B12 deficiency (0%, 5%, 40%; $p < 0.001$) were concentrated in the >36-month group. Duration of PPI use correlated inversely with magnesium ($r = −0.61$), iron ($r = −0.74$), transferrin saturation ($r = −0.82$) and vitamin B12 ($r = −0.78$; all $p < 0.001$), and remained an independent predictor of each parameter after adjustment for age, sex, BMI, daily dose and creatinine. Each additional year of PPI use increased the adjusted odds of hypomagnesaemia 2.8-fold and of vitamin B12 deficiency 4.3-fold.

Conclusion: PPI therapy is associated with a graded, duration-dependent decline in serum magnesium, iron stores and vitamin B12, with clinically significant deficiency emerging predominantly after three years of use. Periodic monitoring of these micronutrients and structured deprescribing should be considered in patients on long-term PPI therapy.

Keywords: Proton pump inhibitors; hypomagnesaemia; iron deficiency; vitamin B12 deficiency; drug-induced micronutrient deficiency; duration of therapy; cross-sectional study.

INTRODUCTION

Proton pump inhibitors (PPIs) irreversibly inhibit the H+/K+-ATPase of the gastric parietal cell and are the most effective agents available for the suppression of gastric acid secretion. Since the introduction of omeprazole in 1989, PPIs have become the mainstay of treatment for gastro-oesophageal reflux disease (GERD), peptic ulcer disease, Helicobacter pylori eradication regimens and gastroprotection during non-steroidal anti-inflammatory drug (NSAID) therapy.1 Their excellent short-term safety profile and ready availability have, however, led to widespread and often prolonged use. Surveys from Europe and North America consistently show that between 40% and 70% of PPI prescriptions lack a clear ongoing indication, and that a large proportion of patients remain on treatment for years without periodic reassessment.2,3 A similar pattern of liberal prescribing, over-the-counter purchase and open-ended continuation is increasingly evident in India, where PPIs are routinely co-prescribed with almost every outpatient prescription.

Gastric acid plays a physiological role in the absorption of several micronutrients. An acidic luminal pH is required to release cobalamin from dietary protein and to reduce ferric iron to the more soluble ferrous form, while magnesium absorption in the colon depends on the transient receptor potential melastatin 6 and 7 (TRPM6/7) channels whose activity is pH sensitive.4,5 Sustained acid suppression is therefore biologically plausible as a cause of hypomagnesaemia, iron deficiency and vitamin B12 deficiency. The first reports of severe, symptomatic PPI-induced hypomagnesaemia appeared in 2006, and the accumulation of further cases prompted the United States Food and Drug Administration to issue a safety communication in 2011 recommending magnesium monitoring in patients anticipated to receive prolonged treatment.6–8 Subsequent meta-analyses of observational studies have confirmed an association between PPI use and hypomagnesaemia with pooled risk estimates of approximately 1.4 to 1.8.9,10

The evidence linking PPIs to vitamin B12 deficiency is similarly consistent. In a large case–control study within an integrated health-care system, two or more years of PPI exposure was associated with a 65% increase in the odds of incident vitamin B12 deficiency, with a clear dose–response gradient.11 Meta-analytical pooling of case–control and cohort data likewise supports a modest but significant effect of long-term acid suppression on cobalamin status.12,13 More recently, population-based data have shown that PPI use for one year or longer is associated with a two-fold increase in the risk of iron deficiency, and that the association strengthens with both dose and duration and diminishes after discontinuation.14,15 Despite this body of literature, several gaps remain. Most of the available data derive from Western populations with dietary patterns, baseline micronutrient status and prescribing practices that differ substantially from those in India, where vitamin B12 deficiency is endemic in predominantly vegetarian communities and iron deficiency anaemia remains the commonest nutritional disorder.16 Very few studies have simultaneously assessed magnesium, iron and cobalamin status in the same cohort of PPI users, and fewer still have examined all three parameters as a function of graded duration of exposure. Such data are important because a clear duration threshold, if one exists, would allow clinicians to target monitoring and deprescribing efforts to the patients at greatest risk.17

The present study was therefore undertaken to compare serum magnesium, iron indices and vitamin B12 levels among adults receiving PPIs for less than 12 months, 12–36 months and more than 36 months, and to quantify the independent relationship between duration of PPI therapy and each biochemical parameter after adjustment for relevant covariates.

MATERIALS AND METHODS

Study design and setting

This hospital-based, analytical cross-sectional study was conducted jointly by the Departments of Pharmacology and Biochemistry, MGM Medical College & LSK Hospital, Kishanganj, Bihar, a tertiary-care teaching hospital serving the Seemanchal region of north-eastern Bihar and adjoining districts of West Bengal. Patients were recruited from the medicine and gastroenterology outpatient departments between August 2025 and June 2026. The study protocol was approved by the Institutional Ethics Committee of MGM Medical College, Kishanganj (Ref. No. _____, dated _____) and was conducted in accordance with the Declaration of Helsinki and the ICMR National Ethical Guidelines for

Biomedical and Health Research involving Human Participants (2017). Written informed consent was obtained from every participant before enrolment.

Study population

Adults aged 18 to 70 years who had been taking any oral PPI (omeprazole, pantoprazole, esomeprazole or rabeprazole) continuously for at least two months, as confirmed from prescription records and pill-count history, were eligible. Patients were excluded if they had chronic kidney disease (estimated glomerular filtration rate <60 mL/min/1.73 m²) or serum creatinine >1.4 mg/dL; known malabsorption syndromes, inflammatory bowel disease, coeliac disease or previous gastric or bariatric surgery; overt gastrointestinal bleeding within the preceding six months; pernicious anaemia or known autoimmune gastritis; chronic liver disease, malignancy, heart failure or uncontrolled diabetes mellitus; current use of loop or thiazide diuretics, metformin, colchicine, cholestyramine or aminoglycosides; intake of magnesium, iron or vitamin B12 supplements or multivitamin preparations within the preceding three months; pregnancy or lactation; or chronic alcohol use. Patients with heavy menstrual bleeding or a documented haemoglobinopathy were also excluded to avoid confounding of the iron indices.

Sample size and grouping

The sample size was estimated for the primary outcome of serum magnesium. Assuming a mean difference of 0.15 mg/dL between the shortest and longest exposure groups with a pooled standard deviation of 0.16 mg/dL (derived from earlier reports of PPI-associated hypomagnesaemia9,18), a two-sided α of 0.05 and power of 80%, the required number was 18 patients per group. Allowing for incomplete data, 20 patients were recruited into each of three duration strata defined a priori: Group I, <12 months; Group II, 12–36 months; and Group III, >36 months of continuous PPI use, giving a total of 60 participants. Consecutive eligible patients were enrolled into each group until its quota was filled.

Data collection

A pre-tested structured proforma was used to record demographic details, body mass index (BMI), indication for PPI therapy, the PPI molecule, daily dose in milligrams, frequency of administration (once or twice daily), total duration of continuous use in months and self-reported adherence (regular, or occasional missed doses defined as missing more than two doses per month). Duration was cross-verified against outpatient records and pharmacy dispensing data wherever available.

Laboratory methods

After an overnight fast of 8–10 hours, 8 mL of venous blood was drawn under aseptic precautions; 2 mL was collected in an EDTA vacutainer for haematological indices and the remainder in a plain vacutainer, allowed to clot, and centrifuged at 3,000 rpm for 10 minutes to separate serum. Serum magnesium (xylidyl blue colorimetric method), serum iron and TIBC (ferrozine method) and creatinine (modified Jaffe kinetic method) were assayed on a fully automated clinical chemistry analyser. Serum ferritin and vitamin B12 were measured by chemiluminescent immunoassay. Haemoglobin and mean corpuscular volume (MCV) were obtained from a five-part automated haematology analyser. Transferrin saturation was calculated as (serum iron ÷ TIBC) × 100. Internal quality control was run daily at two levels and the laboratory participates in an external quality assurance programme. All samples were processed within two hours of collection.

Operational definitions

Hypomagnesaemia was defined as serum magnesium <1.7 mg/dL.9 Iron deficiency was defined as transferrin saturation <20% and/or serum ferritin <30 ng/mL.19 Vitamin B12 status was categorised as deficient (<200 pg/mL), borderline (200–300 pg/mL) or normal (>300 pg/mL).11,20 Anaemia was defined according to World Health Organization criteria as haemoglobin <13 g/dL in men and <12 g/dL in non-pregnant women.21

Statistical analysis

Data were entered into Microsoft Excel and analysed using Python 3.12 with the SciPy (version 1.17) and statsmodels libraries. Continuous variables are expressed as mean ± standard deviation (SD) and categorical variables as frequency (percentage). Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test. Continuous variables were compared across the three duration groups by one-way analysis of variance (ANOVA) with Tukey's honestly significant difference test for post-hoc pairwise comparisons; the Kruskal–Wallis test was applied in parallel to confirm the robustness of results where distributional assumptions were doubtful. Categorical variables were compared using the chi-square test, or Fisher's exact test when any expected cell frequency was less than five. The relationship between duration of PPI use (as a continuous variable) and each biochemical parameter was assessed by Pearson's correlation coefficient, and by multivariable linear regression adjusted for age, sex, BMI, daily PPI dose and serum creatinine. Multivariable logistic regression, adjusted for age and sex, was used to estimate the odds ratio (OR) of hypomagnesaemia and vitamin B12 deficiency per 12-month increment in PPI duration; a model for iron deficiency could

not be fitted because of complete separation (no case occurred in Groups I or II). A two-tailed p value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Sixty patients (29 men, 31 women) with a mean age of 50.6 ± 6.9 years (range 33–67 years) were studied; 51 patients (85.0%) were between 41 and 60 years of age. The mean BMI was 25.8 ± 2.4 kg/m². The commonest indications for PPI therapy were peptic ulcer disease (31.7%) and NSAID gastroprotection (31.7%), followed by GERD (21.7%) and chronic gastritis or non-ulcer dyspepsia (15.0%). Esomeprazole (30.0%) and rabeprazole (28.3%) were the most frequently used molecules; 45 patients (75.0%) took the drug once daily and the modal daily dose was 40 mg. Adherence was regular in 49 patients (81.7%). The mean duration of PPI use was 6.7 ± 2.2 months in Group I, 23.7 ± 5.9 months in Group II and 59.7 ± 13.1 months in Group III (overall range 2–81 months). The three groups were well matched for age, sex, BMI, indication, PPI molecule, daily dose, dosing frequency, adherence and serum creatinine (Table 1).

Table 1. Baseline demographic, clinical and treatment characteristics of the study participants according to duration of PPI use (n = 60)

Characteristic	Group I <12 months (n = 20)	Group II 12–36 months (n = 20)	Group III >36 months (n = 20)	p value
Age (years), mean ± SD	47.9 ± 7.2	51.9 ± 7.2	52.1 ± 5.8	0.095†
Sex, n (%)				0.420‡
Male	12 (60.0)	9 (45.0)	8 (40.0)	
Female	8 (40.0)	11 (55.0)	12 (60.0)	
BMI (kg/m²), mean ± SD	25.7 ± 2.7	26.0 ± 2.8	25.7 ± 1.8	0.881†
Indication, n (%)				0.722‡
Peptic ulcer disease	6 (30.0)	8 (40.0)	5 (25.0)	
NSAID gastroprotection	6 (30.0)	7 (35.0)	6 (30.0)	
GERD	5 (25.0)	4 (20.0)	4 (20.0)	
Chronic gastritis/dyspepsia	3 (15.0)	1 (5.0)	5 (25.0)	
PPI molecule, n (%)				0.300‡
Esomeprazole	3 (15.0)	9 (45.0)	6 (30.0)	
Rabeprazole	5 (25.0)	6 (30.0)	6 (30.0)	
Omeprazole	5 (25.0)	3 (15.0)	5 (25.0)	
Pantoprazole	7 (35.0)	2 (10.0)	3 (15.0)	
Daily dose, n (%)				0.751‡
20 mg	8 (40.0)	5 (25.0)	9 (45.0)	
40 mg	10 (50.0)	12 (60.0)	9 (45.0)	
80 mg	2 (10.0)	3 (15.0)	2 (10.0)	
Twice-daily dosing, n (%)	4 (20.0)	7 (35.0)	4 (20.0)	0.449‡
Regular adherence, n (%)	16 (80.0)	17 (85.0)	16 (80.0)	0.895‡
Duration of PPI use (months), mean ± SD	6.7 ± 2.2	23.7 ± 5.9	59.7 ± 13.1	<0.001†
Serum creatinine (mg/dL), mean ± SD	0.89 ± 0.15	0.86 ± 0.10	0.88 ± 0.10	0.811†

Biochemical parameters across duration groups

Table 2 summarises the biochemical and haematological findings. Every parameter reflecting magnesium, iron and cobalamin status showed a graded decline with increasing duration of PPI exposure. Mean serum magnesium fell from 2.02 ± 0.16 mg/dL in Group I to 1.90 ± 0.13 mg/dL in Group II and 1.76 ± 0.15 mg/dL in Group III ($F = 15.3$, $p < 0.001$). Post-hoc analysis showed that Group III differed significantly from both Group I (mean difference −0.26 mg/dL, 95% CI −0.37 to −0.15; $p < 0.001$) and Group II (−0.15 mg/dL, 95% CI −0.26 to −0.03; $p = 0.008$), while the difference between Groups I and II approached but did not reach significance ($p = 0.052$).

Serum iron declined from 100.0 ± 19.1 µg/dL to 90.3 ± 12.0 µg/dL and 63.3 ± 11.8 µg/dL across the three groups ($F = 33.6$, $p < 0.001$), with Group III significantly lower than both other groups ($p < 0.001$ for each) and no significant difference between Groups I and II ($p = 0.102$). Serum ferritin showed a significant stepwise fall at every level (80.6 ± 16.3, 61.0 ±

18.4 and 38.7 ± 20.8 ng/mL; F = 25.4, p < 0.001; all pairwise p ≤ 0.004), as did transferrin saturation (31.0 ± 5.6%, 26.9 ± 3.3% and 17.4 ± 3.9%; F = 50.2, p < 0.001; all pairwise p ≤ 0.013). TIBC rose reciprocally, being significantly higher in Group III than in Groups I and II (p ≤ 0.001). Serum vitamin B12 decreased from 392.5 ± 56.4 pg/mL to 310.6 ± 61.9 pg/mL and 219.0 ± 73.7 pg/mL (F = 36.3, p < 0.001), with each group differing significantly from the others (all pairwise p ≤ 0.001). Haemoglobin was modestly lower in Group III (12.8 ± 0.9 g/dL) than in Groups I and II (13.4 ± 0.9 and 13.4 ± 0.8 g/dL; ANOVA p = 0.046), although no individual pairwise comparison reached significance after Tukey adjustment. MCV did not differ between groups. Kruskal–Wallis tests yielded the same conclusions for every parameter. The distributions of the three primary analytes are shown in Figure 1.

Table 2. Serum magnesium, iron indices, vitamin B12 and haematological parameters according to duration of PPI use

Parameter (mean ± SD)	Group I <12 months (n = 20)	Group II 12–36 months (n = 20)	Group III >36 months (n = 20)	p value†
Serum magnesium (mg/dL)	2.02 ± 0.16	1.90 ± 0.13	1.76 ± 0.15 a,b	<0.001
Serum iron (µg/dL)	100.0 ± 19.1	90.3 ± 12.0	63.3 ± 11.8 a,b	<0.001
Serum ferritin (ng/mL)	80.6 ± 16.3	61.0 ± 18.4 a	38.7 ± 20.8 a,b	<0.001
TIBC (µg/dL)	323.0 ± 21.8	336.2 ± 25.8	368.2 ± 31.6 a,b	<0.001
Transferrin saturation (%)	31.0 ± 5.6	26.9 ± 3.3 a	17.4 ± 3.9 a,b	<0.001
Vitamin B12 (pg/mL)	392.5 ± 56.4	310.6 ± 61.9 a	219.0 ± 73.7 a,b	<0.001
Haemoglobin (g/dL)	13.4 ± 0.9	13.4 ± 0.8	12.8 ± 0.9	0.046
MCV (fL)	85.3 ± 3.8	86.7 ± 2.7	86.9 ± 3.5	0.281

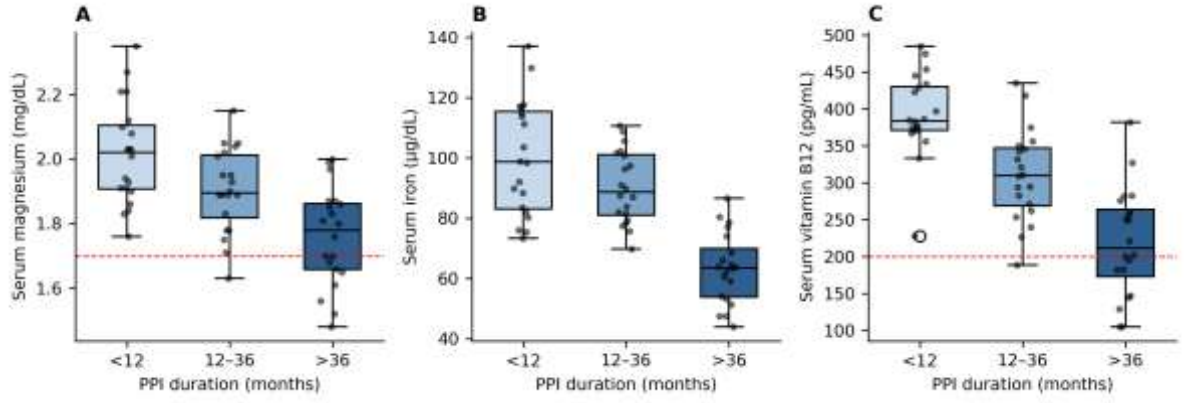

Figure 1. Box-and-whisker plots of (A) serum magnesium, (B) serum iron and (C) serum vitamin B12 according to duration of PPI use. Boxes show the median and interquartile range, whiskers the range, and individual points the values for each patient. Dashed red lines indicate the cut-offs for hypomagnesaemia (1.7 mg/dL) and vitamin B12 deficiency (200 pg/mL).

Prevalence of micronutrient deficiency

Overall, 8 patients (13.3%) had hypomagnesaemia, 16 (26.7%) had iron deficiency and 9 (15.0%) had vitamin B12 deficiency, while a further 19 (31.7%) had borderline B12 values. No patient in Group I had any of the three deficiencies. In Group II, one patient each had hypomagnesaemia and vitamin B12 deficiency, and eight (40.0%) had borderline B12 levels. In Group III, hypomagnesaemia was present in 7 patients (35.0%), iron deficiency in 16 (80.0%) and vitamin B12 deficiency in 8 (40.0%), with a further 10 (50.0%) in the borderline range, so that only 2 patients (10.0%) in this group had a normal vitamin B12 level (Table 3, Figure 2). Compared with all patients treated for 36 months or less, those in Group III had markedly higher odds of hypomagnesaemia (OR 21.0, 95% CI 2.4–186; Fisher p = 0.001) and vitamin B12 deficiency (OR 26.0, 95% CI 3.0–226; Fisher p < 0.001), and iron deficiency occurred exclusively in this group (Fisher p < 0.001). Sixteen of the 20 patients in Group III (80.0%) had at least one deficiency, and 10 (50.0%) had two or more, whereas only 2 of 40 patients treated for 36 months or less (5.0%) had any deficiency. Anaemia by WHO criteria was present in 5 patients (8.3%; 1 man and 4 women), 4 of whom were in Group III.

Table 3. Prevalence of hypomagnesaemia, iron deficiency and vitamin B12 deficiency according to duration of PPI use

Status, n (%)	Group I	Group II	Group III	p value†

	<12 months (n = 20)	12–36 months (n = 20)	>36 months (n = 20)	
Hypomagnesaemia (Mg <1.7 mg/dL)	0 (0.0)	1 (5.0)	7 (35.0)	0.002
Iron deficiency (TSAT <20% and/or ferritin <30 ng/mL)	0 (0.0)	0 (0.0)	16 (80.0)	<0.001
Vitamin B12 status				<0.001
Normal (>300 pg/mL)	19 (95.0)	11 (55.0)	2 (10.0)	
Borderline (200–300 pg/mL)	1 (5.0)	8 (40.0)	10 (50.0)	
Deficient (<200 pg/mL)	0 (0.0)	1 (5.0)	8 (40.0)	
Any deficiency (≥1 of the three)	0 (0.0)	2 (10.0)	16 (80.0)	<0.001
Two or more deficiencies	0 (0.0)	0 (0.0)	10 (50.0)	<0.001

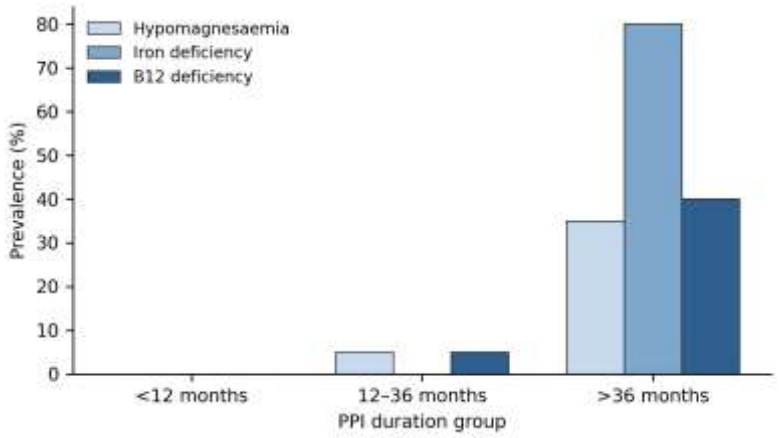

Figure 2. Prevalence of hypomagnesaemia, iron deficiency and vitamin B12 deficiency in each duration group.

Correlation and regression analysis

When duration of PPI use was analysed as a continuous variable, it correlated strongly and inversely with serum magnesium ($r = -0.61$), serum iron ($r = -0.74$), ferritin ($r = -0.68$), transferrin saturation ($r = -0.82$) and vitamin B12 ($r = -0.78$), and positively with TIBC ($r = 0.66$); all $p < 0.001$ (Figure 3). A weaker inverse correlation was observed with haemoglobin ($r = -0.28$, $p = 0.031$), and no correlation with MCV ($r = 0.13$, $p = 0.33$) or serum creatinine ($r = -0.05$, $p = 0.71$). Serum magnesium and vitamin B12 were positively correlated with each other ($r = 0.53$, $p < 0.001$). In contrast, neither daily dose (Spearman $\rho = 0.08$, 0.13 and 0.05 for magnesium, iron and B12 respectively; all $p > 0.3$) nor twice-daily dosing, PPI molecule (ANOVA $p = 0.20$, 0.65 and 0.31) or sex was associated with any of the three analytes.

In multivariable linear regression adjusted for age, sex, BMI, daily dose and serum creatinine (Table 4), duration of PPI use remained a significant independent predictor of every parameter. Each additional month of therapy was associated with a decrease of 0.005 mg/dL in serum magnesium, 0.70 µg/dL in serum iron, 0.72 ng/mL in ferritin, 0.26% in transferrin saturation and 3.1 pg/mL in vitamin B12 (all $p < 0.001$); expressed per year, these correspond to declines of approximately 0.06 mg/dL, 8.3 µg/dL, 8.7 ng/mL, 3.1% and 37 pg/mL respectively. Duration alone explained a substantial proportion of the variance in each outcome (adjusted models $R^2 = 0.40$ to 0.69). Male sex was independently associated with higher ferritin, and higher daily dose with marginally lower ferritin; no other covariate was significant. In logistic regression adjusted for age and sex, each 12-month increment in PPI duration increased the odds of hypomagnesaemia 2.8-fold (OR 2.77, 95% CI 1.45–5.29; $p = 0.002$) and of vitamin B12 deficiency 4.3-fold (OR 4.30, 95% CI 1.64–11.27; $p = 0.003$).

Table 4. Multivariable linear regression of serum magnesium, iron indices and vitamin B12 on duration of PPI use, adjusted for age, sex, BMI, daily PPI dose and serum creatinine

Dependent variable	Unadjusted r with duration	Adjusted β per month of PPI use (95% CI)	p value	Model R^2
Serum magnesium (mg/dL)	−0.61	−0.0048 (−0.0065 to −0.0032)	<0.001	0.40
Serum iron (µg/dL)	−0.74	−0.70 (−0.86 to −0.53)	<0.001	0.58
Serum ferritin (ng/mL)	−0.68	−0.72 (−0.91 to −0.54)	<0.001	0.62
Transferrin saturation (%)	−0.82	−0.26 (−0.30 to −0.21)	<0.001	0.69
Vitamin B12 (pg/mL)	−0.78	−3.06 (−3.75 to −2.37)	<0.001	0.64

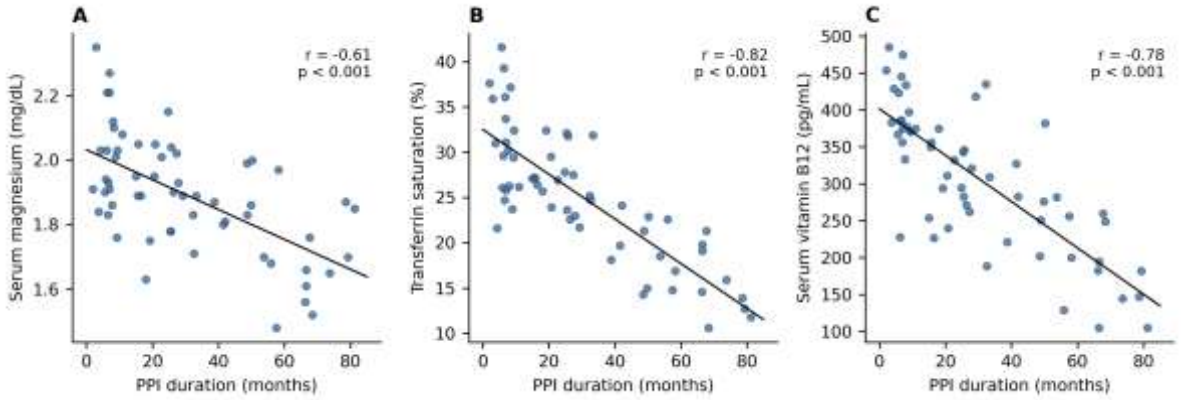

Figure 3. Scatter plots with fitted linear regression lines showing the relationship between duration of PPI use (months) and (A) serum magnesium, (B) transferrin saturation and (C) serum vitamin B12 in all 60 patients.

DISCUSSION

In this cross-sectional study of 60 adults on continuous PPI therapy, we found a consistent, graded and statistically robust decline in serum magnesium, iron stores, transferrin saturation and vitamin B12 with increasing duration of exposure. Patients treated for less than one year had entirely normal values, those treated for one to three years showed early but largely subclinical reductions, and those treated for more than three years had a high burden of frank deficiency: more than one-third had hypomagnesaemia, four-fifths had biochemical iron deficiency and 90% had either deficient or borderline vitamin B12 levels. Duration of therapy remained a strong independent predictor of each parameter after adjustment for age, sex, BMI, dose and renal function, whereas neither the dose nor the specific PPI molecule had any discernible effect. Taken together, these findings support a cumulative, time-dependent effect of gastric acid suppression on micronutrient homeostasis.

Magnesium

The mean serum magnesium of 1.76 mg/dL and the 35% prevalence of hypomagnesaemia in patients treated for more than three years are consistent with the case series of Epstein et al., Cundy and Dissanayake and Hoorn et al., in which symptomatic hypomagnesaemia developed after a median of five or more years of PPI use and recurred promptly on rechallenge.6,7,22 Population-based analyses have confirmed a smaller but significant effect in unselected users; in the Rotterdam Study, PPI use was associated with a 2-fold increase in the odds of hypomagnesaemia, and the risk was highest in those with concomitant loop diuretic use.23 Meta-analyses by Park et al. and Cheungpasitporn et al. reported pooled odds ratios of 1.4 to 1.8.9,10 The mechanism is believed to be impaired active transcellular magnesium absorption through TRPM6/7 channels in the colon, whose function is reduced at higher luminal pH, coupled with a failure of renal compensation because urinary magnesium wasting is minimal in affected patients.5,8 The strong correlation we observed between duration and magnesium, and the near-absence of hypomagnesaemia before three years, mirror the FDA's observation that most reported cases occurred after at least one year of therapy.8,24 We deliberately excluded patients on diuretics and those with renal impairment, and serum creatinine did not differ between groups, so the association is unlikely to be explained by renal magnesium loss.

Iron

Iron deficiency was the most striking finding, being absent in all 40 patients treated for 36 months or less and present in 80% of those treated for longer. Serum iron, ferritin and transferrin saturation all fell, and TIBC rose, in a clear stepwise pattern. These observations agree with the large case–control study of Lam et al., in which two or more years of PPI use was associated with a 2.5-fold increase in the odds of iron deficiency, with the association strengthening with higher daily dose and longer exposure and weakening after discontinuation,14 and with the population-based study of Tran-Duy et al., which found a doubling of risk after one year of use.15 Sarzynski et al. similarly documented progressive falls in haemoglobin and MCV over a year of PPI therapy.25 The reduction in gastric acidity impairs the conversion of dietary ferric iron to the absorbable ferrous form and reduces the release of iron from food; this mechanism was elegantly demonstrated by Hutchinson et al., who showed that PPI therapy suppressed the absorption of non-haem iron in patients with hereditary haemochromatosis,26 and by the observation that iron-deficient patients on omeprazole respond suboptimally to oral ferrous sulphate.27 The clinical relevance of this effect is amplified in the Indian setting, where dietary iron is predominantly non-haem and baseline iron reserves are already marginal in a large proportion of adults.19 Notably, although the biochemical signature of iron deficiency was nearly universal in the long-term group, the fall in haemoglobin

was modest and anaemia was present in only one-fifth of them, indicating that depletion of iron stores precedes anaemia and that measurement of ferritin and transferrin saturation, rather than haemoglobin alone, is required for its detection.

Vitamin B12

Serum vitamin B12 fell by approximately 37 pg/mL for each year of PPI use, and the odds of frank deficiency increased more than four-fold per year. Marcuard et al. first showed in 1994 that two weeks of omeprazole markedly reduced the absorption of protein-bound cyanocobalamin,28 and subsequent observational studies in older adults have consistently shown lower cobalamin levels or higher methylmalonic acid in chronic users of acid-suppressing drugs.29,30 In the Kaiser Permanente case–control study, two or more years of PPI use was associated with an odds ratio of 1.65 for vitamin B12 deficiency, with a stronger association at doses above 1.5 tablets per day.11 Our estimate of effect is larger, which may reflect the substantially lower background B12 status of the Indian population: a large north Indian survey found that nearly half of apparently healthy adults were deficient, attributable mainly to vegetarian diets and low intake of animal protein.16 In such a population, the additional malabsorption imposed by acid suppression may be sufficient to tip a large proportion of long-term users into deficiency. Increased small-intestinal bacterial overgrowth during PPI therapy, which competes for luminal cobalamin, may be an additional contributing mechanism.31 Because a deficit of cobalamin can produce irreversible neurological injury before haematological changes appear, and MCV was normal in almost all our patients, we believe that measurement of serum vitamin B12 should be part of routine surveillance in long-term PPI users in India.

Duration versus dose

An important observation is that duration, rather than dose, frequency or molecule, was the sole treatment-related determinant of micronutrient status in our cohort. Although some previous studies have reported dose–response relationships,11,14 the range of doses in routine practice is narrow and maximal acid suppression is achieved with standard once-daily dosing, so that the cumulative time under acid suppression is likely to be the more relevant exposure metric. The absence of any deficiency before one year and the emergence of most deficiencies after three years suggest a threshold effect that has practical implications: it is consistent with current expert guidance that PPI therapy should be reviewed and, wherever possible, stepped down or stopped once the acute indication has resolved,17,32 and it supports periodic biochemical monitoring in those for whom long-term treatment is unavoidable, such as patients with Barrett's oesophagus, severe erosive oesophagitis or ongoing NSAID or antiplatelet therapy with high gastrointestinal bleeding risk.4

Strengths and limitations

The strengths of this study include the simultaneous assessment of three micronutrients in the same patients, the use of a comprehensive iron panel rather than haemoglobin alone, careful exclusion of the major alternative causes of each deficiency, verification of duration against prescription records, and the use of both categorical and continuous analytic approaches with adjustment for confounders. Several limitations must be acknowledged. The cross-sectional design permits inference of association but not causation, and we cannot exclude the possibility that patients who remain on PPIs for years differ systematically from short-term users in ways that influence micronutrient status. No control group of non-users was included, although the entirely normal values in the short-duration group serve as an internal reference. The sample of 60 patients, while adequately powered for the primary outcome, yields wide confidence intervals for the odds ratios and precludes finer stratification. Dietary intake, in particular vegetarian status, was not quantified and may have contributed to the low B12 values; likewise, measurement of methylmalonic acid or homocysteine, which would have improved the specificity of cobalamin assessment, was not available. Ionised magnesium and urinary magnesium excretion were not measured. Finally, the single-centre setting in a region with a high background prevalence of nutritional deficiency may limit generalisability. A prospective cohort with baseline measurements before PPI initiation, serial sampling and a matched non-user comparison group would be required to confirm the temporal relationships suggested here.

CONCLUSION

Long-term PPI therapy is associated with a duration-dependent decline in serum magnesium, iron stores and vitamin B12 that is independent of dose, molecule, age, sex, BMI and renal function. Deficiency is uncommon within the first year, begins to emerge between one and three years, and becomes highly prevalent thereafter, with most patients treated for more than three years having at least one deficiency and half having two or more. These findings reinforce the need to prescribe PPIs for the shortest effective duration, to reassess the indication periodically, and to monitor serum magnesium, iron indices and vitamin B12 in patients who require treatment beyond one year, particularly in populations with marginal baseline nutritional reserves.

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DECLARATIONS

Consent: Written informed consent was obtained from all participants. Funding: None. Conflicts of interest: None declared. Author contributions: NS conceived and designed the study, supervised patient recruitment and data collection, performed the statistical analysis and drafted the manuscript. SG supervised the laboratory analyses, contributed to data interpretation and critically revised the manuscript. Both authors approved the final version and are accountable for all aspects of the work. Data availability: The de-identified dataset is available from the corresponding author on reasonable request.

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