ORIGINAL RESEARCH

Bagcilar Med Bull 2026;11(3):279-289
DOI: 10.4274/BMB.galenos.2026.81994

Retrospective Analysis of Vitamin B12 and Folic Acid Levels Before and After Antiepileptic (Anticonvulsant) Treatment in Patients with Neuropathic Pain

Nöropatik Ağrı Nedeniyle Antiepileptik (Antikonvülzan) Tedavi Alan Hastalarda B12 ve Folik Asit (Folat) Düzeyinin Tedavi Öncesi ve Sonrası Retrospektif İncelenmesi

Deniz Öke, Zeynel Karakullukçuoğlu

University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, Department of Physical Medicine and Rehabilitation, İstanbul, Turkey

Abstract

Objective: The aim of this study was to investigate the effects of commonly prescribed antiepileptic drugs (pregabalin and gabapentin) on serum vitamin B12 and folate levels in patients with neuropathic pain (NP).

Method: This retrospective study was conducted by reviewing the electronic records of 96 adult patients diagnosed with NP who received treatment with pregabalin or gabapentin between 1 June 2024 and 31 December 2024. Ethics committee approval was obtained on 8 January 2025, and data access and statistical analyses were initiated after this date. Serum vitamin B12, folate, and hematological parameters were obtained from routine laboratory tests performed before antiepileptic drug initiation and from the first available follow-up tests conducted at least three months after treatment initiation. Statistical analyses included the Wilcoxon signed-rank test and paired-samples t-test, as appropriate for within-patient comparisons.

Results: Of 287 potentially eligible patients screened, 96 met the eligibility criteria and were included in the final analysis. Compared with baseline values, post-treatment vitamin B12 levels were lower (from 416.0 pg/mL to 282.0 pg/mL, p=0.001), and folate levels also decreased (from 8.15 ng/mL to 7.45 ng/mL, p=0.003). In subgroup analyses, patients receiving pregabalin showed statistically significant

Öz

Amaç: Bu çalışmanın amacı, nöropatik ağrı (NP) tedavisinde sıklıkla kullanılan antiepileptik ilaçların (pregabalin ve gabapentin) serum vitamin B12 ve folat düzeyleri üzerindeki etkilerini araştırmaktır.

Yöntem: Bu retrospektif çalışma, 1 Haziran 2024 ile 31 Aralık 2024 tarihleri arasında pregabalin veya gabapentin tedavisi gören ve NP tanısı konmuş 96 yetişkin hastanın elektronik kayıtlarının incelenmesiyle gerçekleştirilmiştir. Etik kurul onayı 8 Ocak 2025 tarihinde alınmış olup, veri erişimi ve istatistiksel analizler bu tarihten sonra başlatılmıştır. Serum B12 vitamini, folat ve hematolojik parametreler, antiepileptik ilaç tedavisi başlamadan önce yapılan rutin laboratuvar testlerinden ve tedavi başlangıcından en az üç ay sonra gerçekleştirilen ilk takip testlerinden elde edildi. İstatistiksel analizlerde, hasta içi eşleştirilmiş karşılaştırmalar için veri dağılımına göre Wilcoxon işaretli sıralar testi veya eşleştirilmiş örneklem t-testi kullanıldı.

Bulgular: Potansiyel olarak uygun görülen 287 hastadan 96'sı uygunluk kriterlerini karşıladı ve nihai analize dahil edildi. Tedavi öncesi değerlerle karşılaştırıldığında, tedavi sonrası B12 vitamini düzeyleri daha düşüktü (416,0 pg/mL'den 282,0 pg/mL'ye, p=0,001) ve folat düzeylerinde de azalma gözlendi (8,15 ng/mL'den 7,45 ng/mL'ye, p=0,003). Alt grup analizlerinde, pregabalin alan hastalarda

Address for Correspondence: Deniz Öke, University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, Department of Physical Medicine and Rehabilitation, İstanbul, Turkey
E-mail: dr_denizoke@hotmail.com **ORCID:** orcid.org/0000-0003-3213-0363
Received: 30.09.2025 **Accepted:** 16.09.2026 **Publication Date:** 29.09.2026

Cite this article as: Öke D, Karakullukçuoğlu Z. Retrospective analysis of vitamin B12 and folic acid levels before and after antiepileptic (anticonvulsant) treatment in patients with neuropathic pain. Bagcilar Med Bull. 2026;11(3):279-289

Abstract

decreases in both vitamin B12 (from 448.0 pg/mL to 292.5 pg/mL, p=0.001) and folate levels (from 7.9 ng/mL to 7.5 ng/mL, p=0.007), while in those treated with gabapentin, significant reductions were observed in vitamin B12 (from 368.0 pg/mL to 273.0 pg/mL, p=0.001) and vitamin D levels (from 23.9 ng/mL to 15.7 ng/mL, p=0.021). Sex-stratified analyses showed significant within-group reductions in vitamin B12 and folate levels in both male and female patients.

Conclusion: Pregabalin and gabapentin, widely used in the treatment of NP, are associated with significant reductions in serum vitamin B12 and folate levels. These findings suggest a potential association between antiepileptic drug use and vitamin level reduction. Routine vitamin monitoring may be considered in clinical practice; however, these observational findings do not establish causality, and further prospective studies are required to confirm these associations.

Keywords: Antiepileptic drugs, folate, gabapentin, neuropathic pain, pregabalin, vitamin B12

Öz

hem B12 vitamini (448,0 pg/mL'den 292,5 pg/mL'ye, p=0,001) hem de folat (7,9 ng/mL'den 7,5 ng/mL'e, p=0,007) düzeylerinde istatistiksel olarak anlamlı düşüşler görülürken, gabapentin ile tedavi edilenlerde B12 vitamini (368,0 pg/mL'den 273,0 pg/mL'ye, p=0,001) ve D vitamini (23,9 ng/mL'den 15,7 ng/mL'ye, p=0,021) düzeylerinde anlamlı düşüşler gözlendi. Cinsiyete göre tabakalandırılmış analizlerde, hem erkeklerde hem de kadınlarda B12 vitamini ve folat düzeylerinde grup içi anlamlı azalmalar saptandı.

Sonuç: NP tedavisinde yaygın olarak kullanılan pregabalin ve gabapentin, serum vitamin B12 ve folat düzeylerinde anlamlı azalma ile ilişkili bulunmuştur. Bu bulgular, antiepileptik ilaç kullanımı ile vitamin seviyesindeki azalma arasında potansiyel bir ilişki-olduğunu düşündürmektedir. Klinik uygulamada rutin vitamin takibi düşünülebilir; ancak bu gözlemsel bulgular nedensellik ilişkisini kanıtlamamaktadır ve bu bağlantıları doğrulamak için daha fazla prospektif çalışma gereklidir.

Anahtar kelimeler: Antiepileptik ilaçlar, folat, gabapentin, nöropatik ağrı, pregabalin, vitamin B12

Introduction

Neuropathic pain (NP) is a chronic condition that arises as a direct consequence of a lesion or disease affecting the somatosensory nervous system. It is characterized by maladaptive changes in neural processing that lead to dysfunctional pain signaling pathways and abnormal excitability of peripheral and central neurons (1-4). With a global prevalence of 2-8%, NP significantly impairs quality of life, reduces functional capacity, and imposes considerable socioeconomic costs on patients and healthcare systems (5-7). Despite the availability of multiple therapeutic options, a large proportion of patients remain undertreated or receive suboptimal management (8,9).

The pathophysiology of NP is highly complex and involves peripheral and central sensitization, maladaptive neuroplasticity, and alterations in neurotransmitter signaling (10,11). These processes contribute to heterogeneous clinical presentations and variable treatment responses, which complicates effective management (4). Current guidelines, including the French recommendations established in 2020 based on the GRADE system, emphasize a stepwise pharmacological approach. First-line therapies include serotonin-norepinephrine reuptake inhibitors such as duloxetine and venlafaxine, tricyclic antidepressants, and gabapentinoids (gabapentin and pregabalin), in addition to topical lidocaine and non-pharmacological methods such as transcutaneous electrical nerve stimulation for peripheral NP (12-14). Second-line strategies often involve combination therapies, botulinum toxin A, or high-concentration capsaicin patches, while third-line approaches include spinal cord stimulation, repetitive transcranial magnetic stimulation, and strong opioids, typically reserved for refractory cases (15,16).

Antiepileptic drugs (AEDs), particularly pregabalin and gabapentin, play a crucial role in NP treatment by modulating excitatory neurotransmission through calcium channel blockade (17,18). However, there is growing evidence that chronic AED therapy may negatively affect nutritional status, particularly by reducing serum vitamin B12 and folate levels (19,20). Vitamin B12 deficiency is known to impair myelin synthesis and nerve conduction, potentially worsening neuropathic symptoms or contributing to cognitive decline (19,20). Similarly, folate is essential for DNA synthesis, repair, and methylation, as well as for neuronal health and regeneration (20). Deficiencies in either vitamin may lead to elevated homocysteine levels, which are associated with neurotoxicity and increased cardiovascular risk (1,21,22).

Gabapentinoids are widely used in NP, and growing evidence suggests that AEDs may influence vitamin metabolism. Therefore, evaluating changes in vitamin B12 and folate levels in this population is clinically important. Monitoring these vitamins before and after therapy may help optimize treatment strategies and prevent secondary complications. Accordingly, this study aimed to investigate the association between AED therapy and changes in serum vitamin B12 and folate levels in patients with NP.

Materials and Methods

Study Design and Participants

This study was designed as a retrospective observational analysis. Electronic medical records of adult patients with NP who received pregabalin or gabapentin between 1 June 2024 and 31 December 2024 were reviewed. Eligible participants were identified through the hospital information system based on diagnosis codes and prescription records. Ethics committee approval was obtained on 8 January 2025 (University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, no: 85, date: 08.01.2025), and data extraction as well as statistical analysis were initiated only after this approval, in accordance with national regulations for retrospective data use. As this was a retrospective study based on previously recorded clinical data, the requirement for obtaining informed consent was waived by the Ethics Committee in accordance with institutional policy and national regulations. The study used anonymized data from routine clinical practice, with no direct patient contact or additional tests, and was conducted in accordance with national regulations governing retrospective chart reviews. No direct patient contact occurred, and no identifiable personal information was used. The study had no prospective interventional component; all data were obtained from pre-existing medical records without any additional visits, laboratory tests, or treatment modifications beyond routine clinical care. Eligible participants were identified through electronic medical records. Inclusion criteria required patients to be between 20 and 75 years of age, to have an NP diagnosis confirmed by the DN-4 questionnaire, and to be receiving AED therapy with pregabalin or gabapentin. Additionally, participants had to have serum vitamin B12 and folate levels measured both before and after AED treatment, with no history of vitamin B12 or folate supplementation during therapy. Patients with comorbid conditions that could potentially influence vitamin status, including diabetes mellitus, liver failure, or chronic kidney disease, were excluded from the study.

Patients who met these criteria and had completed at least six months of AED therapy were included in the analysis. Exclusion criteria were: Presence of systemic diseases (e.g., diabetes mellitus, hepatic or renal insufficiency), prior or concurrent vitamin supplementation, and missing laboratory measurements at baseline or follow-up.

Because several medical conditions and commonly used medications may independently influence serum vitamin B12 and folate concentrations, potential confounders were carefully reviewed through electronic medical records. Patients receiving medications known to reduce vitamin B12 or folate levels—such as metformin, proton pump inhibitors, H2-blockers, or long-term antifolate agents—were excluded from the study. Additionally, patients with clinically significant hypothyroidism, untreated hyperlipidemia, or other systemic conditions that could alter vitamin absorption or metabolism were excluded unless laboratory data confirmed stable disease and adequate control prior to initiation of antiepileptic therapy. These measures were implemented to minimize confounding and improve internal validity.

Data Collection and Laboratory Measurements

Patient data, including demographic information (age, sex), comorbidities, type of AED used (pregabalin or gabapentin), daily drug dose, and duration of therapy, were retrospectively extracted from hospital records.

Biochemical analyses included serum vitamin B12, folate, and vitamin D levels, measured at two time points: (1) At baseline, defined as the laboratory assessment performed within 0-4 weeks before AED initiation, and (2) at the first available follow-up measurement obtained at least 3 months after treatment initiation. Because of the retrospective design, the timing of follow-up tests was not protocol-driven but reflected routine clinical practice; therefore, follow-up intervals varied between patients and were not standardized. The reported median treatment duration of 16.5 months refers to the total duration of AED use at the time of data extraction and does not represent the fixed interval between baseline and follow-up laboratory measurements. Hematological parameters such as hemoglobin, hematocrit, mean corpuscular volume (MCV), red blood cell count, platelet count, mean platelet volume, plateletcrit (PCT), white blood cell (WBC) count, and differential leukocyte counts were also recorded when available. For each laboratory parameter, paired analyses were restricted to patients with both baseline and post-treatment measurements; sex- and drug-specific subgroup analyses were conducted within the corresponding analyte-specific paired cohort.

The reference ranges applied in this study were 180-920 pg/mL for vitamin B12, 3-17 ng/mL for folic acid (folate), and 20-50 ng/mL for vitamin D. These intervals reflect widely accepted clinical laboratory standards used to evaluate vitamin status in adults. The chosen cut-off values are consistent with commonly reported ranges in the literature,

where serum vitamin B12 concentrations below 200 pg/mL indicate deficiency and values between 200-300 pg/mL suggest borderline status (23,24). Similarly, folate levels below 3 ng/mL are typically considered deficient, while levels above 4 ng/mL are regarded as adequate for normal hematopoietic and neurological function (1,25). For vitamin D, concentrations below 20 ng/mL are generally defined as deficient, whereas levels between 20-50 ng/mL are considered sufficient for bone and musculoskeletal health (26,27). These standardized thresholds ensure comparability with prior studies and allow for reliable interpretation of laboratory findings in the context of NP and AED therapy.

Serum vitamin D concentrations were measured using a standardized chemiluminescence immunoassay. Vitamin B12 and folate levels were analyzed using immunoassay-based methods, and all reference intervals corresponded to manufacturer-provided ranges validated by the hospital's central biochemistry laboratory. All assays were performed according to the manufacturer's instructions, following routine internal quality control protocols.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as median (minimum-maximum) values, and categorical variables were presented as frequencies and percentages. The normality of continuous variables was assessed using the Shapiro-Wilk test. For paired comparisons of baseline and post-treatment values, the Wilcoxon signed-rank test was used for non-normally distributed variables, whereas the paired-sample t-test was applied when normality assumptions were met. For paired comparisons of baseline and post-treatment values in the overall cohort and within sex- and drug-specific subgroups, the Wilcoxon signed-rank test was used for non-normally distributed variables, whereas the paired-samples t-test was applied when the normality assumption was met. The p-values reported for the sex-stratified analyses represent within-sex paired comparisons. No formal between-sex comparison of individual change scores was performed; therefore, numerical differences between male and female strata were not interpreted as evidence of sex-specific treatment effects. In addition to p-values, effect sizes for paired non-parametric comparisons were calculated using the rank-biserial correlation (rrb). For descriptive purposes, the percentage change in group medians was calculated for vitamin B12 and folate as [(post-treatment group median–baseline group median)/baseline group median] ×100. Negative values indicate a reduction. These values were calculated from the group medians and do not represent the median of individual patient-level percentage changes; they were not used for inferential testing. Because the primary outcomes were predefined, no formal multiple-comparison correction was applied; however, secondary analyses were interpreted cautiously. A p-value <0.05 was considered statistically significant. Because this was an observational retrospective study, no a priori sample size calculation was performed. Subgroup analyses were exploratory in nature, and statistical power may be reduced in smaller strata, particularly in gender- and drug-specific comparisons. The analyses were primarily designed to characterize within-patient changes in vitamin levels. Multivariable regression was not prespecified, and the available sample size was considered modest for simultaneous adjustment for multiple potential confounders. Therefore, the subgroup analyses were considered exploratory, and the observed associations were interpreted cautiously without attributing the changes independently to drug exposure.

Results

During the study period, a total of 287 patients with neuropathic pain who had been prescribed pregabalin or gabapentin between 1 June 2024 and 31 December 2024 were screened for eligibility through the electronic medical record system. Of these, 191 patients were excluded according to the predefined eligibility criteria: 64 because of missing baseline vitamin B12 and/or folate data, 58 because of missing post-treatment vitamin B12 and/or folate data, 23 because they received vitamin B12 or folate supplementation during the treatment period, 27 because of comorbid conditions potentially affecting vitamin B12 or folate metabolism, 14 because of concomitant medications known to interfere with vitamin B12 or folate status, and 5 because they did not meet other eligibility criteria. After these exclusions, 96 patients fulfilled all eligibility criteria and were included in the final analysis, of whom 59 received pregabalin and 37 received gabapentin (Figure 1).

Demographic and clinical characteristics of patients are presented in Table 1. The median age of the included patients was 61.0 years (range, 20-75 years). The cohort consisted of 67 males (69.8%) and 29 females (30.2%). Regarding comorbidities, 30.2% had no comorbidity, while 21.9% had multiple comorbid conditions. The most common individual comorbidities were hypertension (13.5%), hypothyroidism (10.4%), and hyperlipidemia (6.3%). In terms of medication, 61.5% of the patients were using pregabalin, and 38.5% were using gabapentin. The

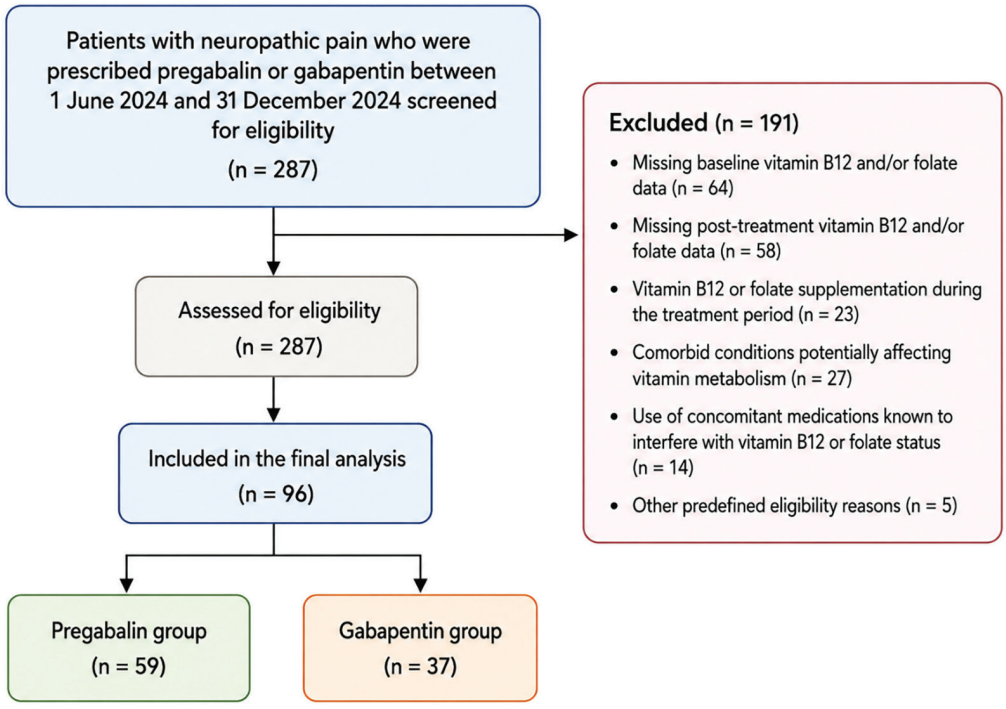

Figure 1. Flow diagram of patient screening, exclusion, and inclusion in the final study population

median drug dose was 300.00 mg/day (25.00-2400.00), and the duration of use was 16.5 months (3.00-149.00). This duration reflects the total exposure to AED therapy at the time of data extraction. Baseline and post-treatment laboratory results demonstrated statistically significant changes in vitamin levels. Other hematological parameters included a median hemoglobin of 13.20 g/dL, hematocrit of 39.80%, MCV of 83.30 fL, and platelet count of 262.00×10^3/µL. Inflammatory and immune cell markers such as WBC, neutrophils, lymphocytes, and others were within expected ranges (Table 1).

The comparison of baseline and post-treatment laboratory values demonstrated statistically significant changes in vitamin B12 and folic acid levels during AED treatment; however, these changes should be interpreted as associations rather than proof of causality. The median vitamin B12 level significantly decreased from 416.00 pg/mL (range: 145.00-1993.00) at baseline to 282.00 pg/mL (117.00-1216.00) after treatment (p=0.001). The median folic acid level declined from 8.15 ng/mL (2.50-22.00) to 7.45 ng/mL (3.40–17.10), also reaching statistical significance (p=0.003). In contrast, the change in vitamin D levels from 20.55 ng/mL (5.00-51.90) at baseline to 19.05 ng/mL (5.70-52.30) after treatment did not reach statistical significance (p=0.122). The percentage changes in group medians were -32.2% for vitamin B12 and -8.6% for folate. The effect size (rank-biserial correlation) indicated a large effect for the decline in vitamin B12 (rrb=0.62) and a moderate effect for the reduction in folate (rrb=0.34) (Table 2). Sex-stratified within-group analyses showed statistically significant reductions in vitamin B12 and folate levels from baseline to post-treatment in both male and female patients. Among males, median vitamin B12 decreased from 430.00 pg/mL (145.00-1757.00) to 273.00 pg/mL (137.00-1216.00), and folic acid from 8.35 ng/mL (2.50-22.00) to 7.60 ng/mL (3.40-17.10), with p-values of 0.001 and 0.028, respectively. A significant reduction was also observed in vitamin D levels among males (p=0.040). In female patients, vitamin B12 levels declined significantly from 407.50 pg/mL (169.00-1993.00) to 311.50 pg/mL (117.00-699.00) (p=0.001), and folic acid dropped from 8.00 ng/mL (3.20-15.00) to 6.60 ng/mL (3.90-14.60) (p=0.038). However, the change in vitamin D levels among female patients, from 19.00 ng/mL (5.00-35.00) at baseline to 20.80 ng/mL (8.00-52.30) after treatment, was not statistically significant (p=0.066). Effect sizes for gender-specific analyses demonstrated a large effect for vitamin B12 decline in males (rrb=0.59) and a moderate effect in females (rrb=0.41) (Table 3). In the comparison of baseline and post-treatment hematological parameters, significant decreases were observed in several

Table 1. Baseline demographic, clinical, and hematological characteristics of the entire study cohort

Parameter (unit)	Median (min-max) or n (%)
Age (years)	61.00 (20.00-75.00)
Gender-male	67 (69.8%)
Gender-female	29 (30.2%)
Comorbidity	
No comorbidity	29 (30.2%)
Hypertension	13 (13.5%)
Hypothyroidism	10 (10.4%)
Hyperlipidemia	6 (6.3%)
Respiratory disease	3 (3.1%)
Cardiac disease	5 (5.2%)
Multiple	21 (21.9%)
Other	8 (8.3%)
Drug type	
Pregabalin	59 (61.5%)
Gabapentin	37 (38.5%)
Drug dose (mg/day)	300.00 (25.00-2400.00)
Duration of use (months)	16.50 (3.00-149.00)
RBC (10^6/µL)	4.78 (3.44-6.16)
Hemoglobin (g/dL)	13.20 (7.90-15.70)
Hematocrit (%)	39.80 (30.70-48.90)
MCV (fL)	83.30 (67.00-100.20)
MCH (pg)	27.20 (21.80-32.40)
MCHC (g/dL)	32.70 (28.10-36.00)
RDW (%)	13.90 (10.90-23.40)
Platelets (10^3/µL)	262.00 (132.00-494.00)
MPV (fL)	9.30 (6.60-13.90)
PDW (%)	13.80 (10.00-20.00)
PCT (%)	0.23 (0.11-0.47)
WBC (10^3/µL)	7.13 (3.10-15.20)
Neutrophils (10^3/µL)	4.40 (1.30-12.30)
Lymphocytes (10^3/µL)	2.00 (0.60-4.40)
Monocytes (10^3/µL)	0.49 (0.18-1.20)
Eosinophils (10^3/µL)	0.12 (0.01-0.66)
Basophils (10^3/µL)	0.02 (0.00-0.27)

RBC: Red blood cell count, MPV: Mean platelet volume, PCT: Plateletcrit, WBC: White blood cell, RDW: Red cell distribution width, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

indices. Platelet counts showed a notable reduction from a median of 264.0×10^3/µL to 239.0×10^3/µL (p=0.033). PCT levels declined significantly from 0.3% to 0.2% (p=0.008). Among leukocyte parameters, the total WBC count decreased from 7250.0/µL to 7005.0/µL (p=0.031), while neutrophil counts declined from 4300.0/µL to 3880.0/µL (p=0.043). No statistically significant changes were detected in red blood cell indices, hemoglobin, hematocrit, lymphocyte counts, or platelet volume indices (p>0.05).

In patients treated with pregabalin, a significant reduction in vitamin B12 levels was observed, with median values decreasing from 448.0 pg/mL at baseline to 292.5 pg/mL post-treatment (p=0.001). Similarly, serum folate concentrations declined from 7.9 ng/mL to 7.5 ng/mL, reaching statistical significance (p=0.007). Hematological analysis revealed a significant decrease in platelet counts, from 283.0×10^3/µL to 240.5×10^3/µL (p=0.037), as well as a reduction in PCT levels from 0.3% to 0.2% (p=0.013). In contrast, no significant changes were noted in vitamin D levels, red blood cell indices, hemoglobin, hematocrit, or leukocyte counts (p>0.05) (Table 4). In patients receiving gabapentin therapy, serum vitamin B12 levels showed a significant decline, with median values decreasing from 368.0 pg/mL at baseline to 273.0 pg/mL after treatment (p=0.001). Vitamin D concentrations also dropped significantly, from 23.9 ng/mL to 15.7 ng/mL (p=0.021). In addition, MCHC demonstrated a slight but statistically significant reduction, from 33.1 g/dL to 32.5 g/dL (p=0.047). No significant changes were observed in folate levels, red blood cell indices, platelet parameters, or leukocyte counts (p>0.05) (Table 5).

Discussion

This study provides observational evidence regarding the association between AED therapy and alterations in vitamin B12 and folate status in patients with NP. Our findings demonstrate statistically significant reductions in both vitamins over the course of AED treatment; however, due to the retrospective design, these changes cannot be interpreted as causal effects.

Specifically, the group median folate level declined from 8.15 ng/mL to 7.45 ng/mL (p=0.003), while the group median vitamin B12 level decreased from 416.0 pg/mL to 282.0 pg/mL (p=0.001). These values correspond to percentage changes in group medians of -8.6% for folate and -32.2% for vitamin B12. Consistent with our findings, Huang et al. (20) also reported significant decreases in serum folate and vitamin B12 levels among epilepsy patients following AED administration. Similar reductions reported in previous studies (20) support the possibility that AEDs may influence vitamin metabolism, but prospective and mechanistic studies are needed to confirm this hypothesis.

The clinical implications of these biochemical changes are noteworthy. Vitamin B12 deficiency is known to cause

Table 2. Comparison of baseline and post-treatment laboratory parameters

Parameter (unit)	Baseline	Post-treatment	p-value
Vitamin B12 (pg/mL)	416.00 (145.00-1993.00)	282.00 (117.00-1216.00)	**0.001**
Folic acid (ng/mL)	8.15 (2.50-22.00)	7.45 (3.40-17.10)	**0.003**
Vitamin D (ng/mL)	20.55 (5.00-51.90)	19.05 (5.70-52.30)	0.122

Data are presented as median (minimum-maximum). For each parameter, paired analyses included patients with both baseline and post-treatment measurements

Table 3. Paired comparison of baseline and post-treatment laboratory parameters stratified by sex

Gender	Parameter (unit)	Baseline	Post-treatment	p-value
Male	Vitamin B12 (pg/mL)	430.00 (145.00-1757.00)	273.00 (137.00-1216.00)	**0.001**
Male	Folic acid (ng/mL)	8.35 (2.50-22.00)	7.60 (3.40-17.10)	**0.028**
Male	Vitamin D (ng/mL)	20.90 (6.50-51.90)	17.90 (5.70-41.00)	**0.040**
Female	Vitamin B12 (pg/mL)	407.50 (16.00-1993.00)	311.50 (117.00-699.00)	**0.001**
Female	Folic acid (ng/mL)	8.00 (3.20-15.00)	6.60 (3.90-14.60)	**0.038**
Female	Vitamin D (ng/mL)	19.00 (5.0-35.0)	20.80 (8.0-50.60)	0.066

Data are presented as median (minimum-maximum). The reported p-values represent paired baseline-to-post-treatment comparisons performed separately within male and female patients. No direct between-sex comparison of individual changes was performed

Table 4. Comparison of baseline and post-treatment parameters in patients receiving pregabalin

Parameter (unit)	Baseline	Post-treatment	p-value
Vitamin B12 (pg/mL)	448.0 (145.0-1993.0)	292.5 (147.0-770.0)	**0.001**
Vitamin D (ng/mL)	19.4 (5.00-51.90)	20.9 (5.70-52.30)	0.904
Folate (ng/mL)	7.9 (2.5-22.0)	7.5 (3.9-17.1)	**0.007**
RBC ($\times 10^6$/µL)	4.5 (3.5-5.5)	4.6 (3.4-5.8)	0.265
Hemoglobin (g/dL)	12.5 (7.9-15.7)	12.9 (9.5-15.7)	0.394
Hematocrit (%)	37.8 (27.9-46.1)	38.8 (31.8-46.6)	0.521
MCV (fL)	86.5 (60.5-100.7)	85.8 (66.9-99.5)	0.739
MCH (pg)	28.4 (17.2-32.7)	28.0 (21.3-33.7)	0.975
MCHC (g/dL)	32.5 (28.5-35.1)	32.6 (29.8-34.8)	0.322
Platelets ($\times 10^3$/µL)	283.0 (176.0-411.0)	240.5 (56.0-442.0)	**0.037**
MPV (fL)	9.9 (1.9-12.7)	9.6 (7.6-13.9)	0.804
PDW (%)	16.0 (11.4-22.8)	15.9 (10.5-17.8)	0.604
PCT (%)	0.3 (0.2-0.4)	0.2 (0.1-0.4)	**0.013**
WBC-total ($\times 10^3$/µL)	7.35 (3.85-13.63)	7.45 (2.89-10.03)	0.143
Neutrophils ($\times 10^3$/µL)	4.34 (1.17-9.46)	4.15 (1.80-6.79)	0.222
Lymphocytes($\times 10^3$/µL)	2.45 (1.06-4.24)	2.35 (0.34-3.55)	0.528

RBC: Red blood cell count, MPV: Mean platelet volume, PCT: Plateletcrit, WBC: White blood cell, RDW: Red cell distribution width, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

peripheral neuropathy, raising the possibility of a paradox in which medications used to alleviate NP may simultaneously exacerbate underlying neuronal dysfunction (28). Indeed, prior studies reported subnormal vitamin B12 levels in approximately 44.8% of patients treated with carbamazepine, suggesting that gabapentinoids may induce comparable metabolic effects (19,29).

Several mechanisms may contribute to AED-related vitamin deficiencies. These include impaired vitamin B12 absorption due to increased intestinal pH, inhibition of folate-metabolizing enzymes, and competitive interference between folate and AEDs at intestinal absorption sites (1,17,19,22). Previous evidence also indicates that long-term AED therapy alters folate and vitamin B12 bioavailability, particularly with phenytoin. Moreover, calcium channel-acting drugs such as pregabalin and gabapentin may inhibit transport mechanisms required for vitamin uptake (30,31).

Sex-stratified analyses showed significant within-group reductions in vitamin B12 and folate levels among both male and female patients. However, individual changes

Table 5. Comparison of baseline and post-treatment parameters in patients receiving gabapentin

Parameter (unit)	Baseline	Post-treatment	p-value
Vitamin B12 (pg/mL)	368.0 (169.0-1000.0)	273.0 (117.0-1216.0)	**0.001**
Vitamin D (ng/mL)	23.9 (7.1-50.6)	15.7 (6.0-33.7)	**0.021**
Folate (ng/mL)	8.9 (3.2-15.6)	7.5 (3.4-14.6)	0.199
RBC ($\times10^6$/µL)	4.5 (4.0-6.8)	4.5 (3.9-6.0)	0.931
Hemoglobin (g/dL)	13.3 (9.4-14.8)	13.4 (9.9-15.6)	0.509
Hematocrit (%)	39.6 (30.9-44.3)	40.8 (31.9-48.1)	0.569
MCV (fL)	88.5 (63.1-96.4)	86.5 (58.4-100.3)	0.280
MCH (pg)	28.8 (20.4-32.7)	28.9 (19.0-32.8)	0.279
MCHC (g/dL)	33.1 (30.2-34.8)	32.5 (31.0-34.4)	**0.047**
Platelets ($\times10^3$/µL)	250.0 (130.0-447.0)	233.0 (128.0-437.0)	0.345
MPV (fL)	9.9 (7.1-13.3)	9.7 (7.8-13.9)	0.959
PDW (%)	16.2 (9.4-16.7)	16.1 (15.0-16.7)	0.988
PCT (%)	0.2 (0.1-0.3)	0.2 (0.1-0.4)	0.352
WBC-total ($\times10^3$/µL)	7.09 (4.29-10.99)	6.68 (4.82-9.47)	0.088
Neutrophils ($\times10^3$/µL)	4.23 (2.29-7.56)	3.71 (2.31-7.10)	0.064
Lymphocytes($\times10^3$/µL)	2.14 (1.19-4.25)	2.16 (1.10-3.03)	0.584

RBC: Red blood cell count, MPV: Mean platelet volume, PCT: Plateletcrit, WBC: White blood cell, RDW: Red cell distribution width, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

were not formally compared between sexes. Therefore, the observed numerical differences in percentage change should not be interpreted as evidence of sex-specific effects or used to support sex-specific supplementation strategies. Future studies should evaluate whether sex modifies these changes through direct comparisons of individual change scores or longitudinal models incorporating a time-by-sex interaction.

Importantly, the present findings do not establish that the observed reductions in vitamin levels are independently attributable to pregabalin or gabapentin. Age and sex may influence vitamin status and therefore remain potential confounders in this retrospective cohort. Although major medical conditions and medications known to interfere with vitamin B12 or folate metabolism were excluded, residual confounding related to age, sex, baseline vitamin status, dietary factors, treatment duration, drug dose, and other unmeasured characteristics cannot be excluded. Accordingly, the observed pre-post differences should be interpreted as associations rather than drug-specific causal effects. Larger prospective studies incorporating multivariable models with changes in vitamin B12 and folate as outcomes and age, sex, drug type, dose, treatment duration, and baseline vitamin concentrations as covariates are needed to determine whether gabapentinoid exposure is independently associated with vitamin depletion.

Given the observed associations, closer monitoring of vitamin B12 and folate levels may be considered in selected high-risk patients undergoing AED therapy for NP; however, routine supplementation or systematic screening cannot be recommended solely on the basis of this single retrospective study. Future prospective and randomized controlled trials are required to determine whether vitamin monitoring and supplementation improve clinical outcomes in NP. Previous reports have described variable vitamin B12 outcomes under long-term AED use, ranging from decreases to normal or even elevated levels. Importantly, deficiencies may arise weeks to months after initiation of therapy (32). Therefore, these findings may suggest a potential role for closer monitoring and individualized supplementation strategies, particularly in high-risk groups such as elderly individuals, malnourished patients, or those with multiple comorbidities (33). Of note, a recent retrospective cohort study reported that repeated gabapentin prescriptions among adults with chronic low back pain were associated with increased risks of dementia and mild cognitive impairment, particularly among adults aged 18-64 years (34). Taken together, these observations highlight the potential clinical relevance of monitoring vitamin status; however, further prospective studies are required before establishing definitive clinical recommendations.

From a health economics perspective, routine supplementation may represent a potentially cost-effective strategy; however, this requires confirmation in future studies. Vitamin B12 and folic acid insufficiency contribute to elevated homocysteine levels, which have been implicated as independent risk factors for cardiovascular, cerebrovascular, and peripheral vascular diseases (35,36). Hyperhomocysteinemia-associated peripheral neuropathy, in combination with vascular complications, substantially increases healthcare costs and patient morbidity (37). Timely supplementation may therefore prevent such complications while simultaneously enhancing treatment outcomes and quality of life.

Study Limitations

This study has some limitations that should be acknowledged. First, the relatively short follow-up period may not adequately reflect long-term alterations in vitamin status associated with AED therapy. Second, functional outcomes and quality-of-life measures were not assessed, which limits the clinical interpretation of the observed biochemical changes. Previous studies have reported a dose-dependent relationship between certain AEDs, including pregabalin and topiramate, and serum vitamin B12 and folate concentrations; however, our study was not designed to evaluate such associations in detail. Additional limitations include the relatively small sample size, the retrospective design, and the use of two different AEDs with varying dosing regimens, which may have introduced heterogeneity and limited the ability to establish a clear dose–response relationship. Although major confounding conditions were screened and patients with uncontrolled systemic diseases or medications known to interfere with vitamin metabolism were excluded, the retrospective design does not fully eliminate the possibility of residual confounding. Furthermore, multivariable regression adjusting simultaneously for age, sex, drug type, drug dose, treatment duration, and baseline vitamin concentrations was not performed. Therefore, the present study cannot determine the independent contribution of gabapentinoid therapy to the observed changes in vitamin levels, and residual confounding remains possible. Chronic illnesses such as hypothyroidism or hyperlipidemia, even when stable, may have subtle effects on vitamin homeostasis, and these factors could contribute to variability in the observed results. Another limitation is the modest sample size, particularly after stratification by drug type and gender. Smaller subgroup sizes reduce statistical power and may limit the robustness of the observed effect sizes and subgroup-specific findings. Therefore, these results should be interpreted with caution, and larger prospective studies are warranted to validate these subgroup differences.

Future randomized controlled trials are warranted to assess the effects of systematic vitamin supplementation on rehabilitation outcomes, functional capacity, and quality of life in patients with NP. Such studies should also determine optimal AED dosing strategies, appropriate timing for supplementation, and identify high-risk patient subgroups. Addressing these aspects would provide more robust evidence to guide clinical practice and inform the development of comprehensive treatment guidelines.

Implications for Future Research

Future research should focus on prospective, large-scale randomized controlled trials to further elucidate the relationship between gabapentinoid therapy and alterations in vitamin B12 and folate levels. Such studies should not only evaluate long-term biochemical changes but also incorporate functional and clinical outcomes, including neuropathic symptom progression, cognitive performance, and quality of life. Investigating potential dose–response relationships, differences between various AED classes, and the impact of treatment duration will provide a more comprehensive understanding of the mechanisms underlying vitamin depletion. Furthermore, studies should aim to identify high-risk subgroups, such as elderly patients, those with nutritional deficiencies, or individuals with multiple comorbidities, to inform targeted supplementation strategies. Incorporating cost-effectiveness analyses and health economic perspectives would also be valuable in incorporating cost-effectiveness analyses and health economic perspectives would also be valuable in shaping evidence-based guidelines for vitamin monitoring in clinical practice; however, further prospective studies are required.

Conclusion

In conclusion, this study demonstrates that AED therapy use is associated with significant reductions in serum vitamin B12 and/or folate levels in patients with NP. These findings suggest a potential relationship between antiepileptic drug use and altered vitamin status. Routine monitoring of vitamin levels may be considered in clinical practice, particularly in high-risk populations; however, further prospective studies are required to confirm these findings and to establish clinical recommendations.

The results further suggest that integrating nutritional assessment and vitamin supplementation into NP management may be beneficial; however, the potential clinical impact of such approaches should be interpreted with caution and requires validation in prospective studies. Such an approach may be consistent with multidisciplinary care models and highlights the importance of considering both pharmacological and nutritional dimensions of patient care.

Establishing systematic vitamin monitoring and supplementation strategies may represent a promising approach in the comprehensive management of NP, although further evidence is needed to support their routine implementation. Ultimately, these findings highlight the interconnected nature of drug therapy, treatment duration, and nutritional status, and support the need for further research in this area. Due to the retrospective design, causality cannot be established, and the findings should be interpreted as associations.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained on 8 January 2025 (University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital, no: 85, date: 08.01.2025), and data extraction as well as statistical analysis were initiated only after this approval, in accordance with national regulations for retrospective data use.

Informed Consent: As this was a retrospective study based on previously recorded clinical data, the requirement for obtaining informed consent was waived by the ethics committee in accordance with institutional policy and national regulations. The study used anonymized data from routine clinical practice, with no direct patient contact or additional tests, and was conducted in accordance with national regulations governing retrospective chart reviews. No direct patient contact occurred, and no identifiable personal information was used.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.Ö., Concept: D.Ö., Z.K., Design: D.Ö., Z.K., Data Collection or Processing: D.Ö., Z.K., Analysis or Interpretation: D.Ö., Z.K., Literature Search: D.Ö., Z.K., Writing: D.Ö., Z.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Zhang Y, Mustieles V, Wang YX, Sun Y, Agudelo J, Bibi Z, et al. Folate concentrations and serum perfluoroalkyl and polyfluoroalkyl substance concentrations in adolescents and adults in the USA (National Health and Nutrition Examination Study 2003-16): an observational study. Lancet Planet Health. 2023;7(6):e449-e458.
2. Bouhassira D, Attal N. Translational neuropathic pain research: a clinical perspective. Neuroscience. 2016;338:27-35.
3. Rosner J, Attal N, Finnerup NB. Clinical pharmacology of neuropathic pain. Int Rev Neurobiol. 2024;179:403-430.
4. Ma YC, Kang ZB, Shi YQ, Ji WY, Zhou WM, Nan W. The complexity of neuropathic pain and central sensitization: exploring mechanisms and therapeutic prospects. J Integr Neurosci. 2024;23(5):89.
5. Soliman N, Kersebaum D, Lawn T, Sachau J, Sendel M, Vollert J. Improving neuropathic pain treatment - by rigorous stratification from bench to bedside. J Neurochem. 2024;168(11):3699-3714.
6. Rahman MM, Jo YY, Kim YH, Park CK. Current insights and therapeutic strategies for targeting TRPV1 in neuropathic pain management. Life Sci. 2024;355:122954.
7. Testa L, Dotta S, Vercelli A, Marvaldi L. Communicating pain: emerging axonal signaling in peripheral neuropathic pain. Front Neuroanat. 2024;18:1398400.
8. Shkodina AD, Bardhan M, Chopra H, Anyagwa OE, Pinchuk VA, Hryn KV, et al. Pharmacological and non-pharmacological approaches for the management of neuropathic pain in multiple sclerosis. CNS Drugs. 2024;38(3):205-224.
9. Boccella S, De Filippis L, Giorgio C, Brandolini L, Jones M, Novelli R, et al. Combination drug therapy for the management of chronic neuropathic pain. Biomolecules. 2023;13(12):1802.
10. Andrejic N, Bozovic I, Moradi H, Tataei R, Knezevic NN. Neuropathic pain management: a focused review of current treatments and novel data from main ongoing clinical trials. Expert Opin Investig Drugs. 2025;34(4):287-299.
11. Tesfaye S, Sloan G, Petrie J, White D, Bradburn M, Young T, et al. Optimal pharmacotherapy pathway in adults with diabetic peripheral neuropathic pain: the OPTION-DM RCT. Health Technol Assess. 2022;26(39):1-100.
12. Liu X, Wei L, Zeng Q, Lin K, Zhang J. The treatment of topical drugs for postherpetic neuralgia: a network meta-analysis. Pain Physician. 2020;23(6):541-551.
13. Moisset X, Bouhassira D, Attal N. French guidelines for neuropathic pain: an update and commentary. Rev Neurol (Paris). 2021;177(7):834-837.
14. Dwajani S, Ravi L, Bindu CM, Prema R. Effect of antiepileptic drugs on serum vitamin B12 and folic acid among children with epilepsy in Bangalore. Biomedical and Pharmacology Journal. 2024;17(1):263-269.
15. Moisset X. Neuropathic pain: evidence based recommendations. Presse Med. 2024;53(2):104232.
16. Freynhagen R, Baron R, Huygen F, Perrot S. Narrative review of the efficacy and safety of the high-concentration (179mg) capsaicin patch in peripheral neuropathic pain with recommendations for clinical practice and future research. Pain Rep. 2025;10(2):e1235.

17. Kathiravan M, Kavitha S, Shanthi R. To determine the effect of long-term antiepileptic drug on the serum folate and vitamin B12 among epileptic patients. Sci Rep. 2021;11(1):4393.

18. Petroianu GA, Aloum L, Adem A. Neuropathic pain: mechanisms and therapeutic strategies. Front Cell Dev Biol. 2023;11:1072629.

19. Safahani M, Aligholi H, Asadi-Pooya AA. Management of antiepileptic drug-induced nutrition-related adverse effects. Neurol Sci. 2020;41(12):3491-3502.

20. Huang HL, Zhou H, Wang N, Yu CY. Effects of antiepileptic drugs on the serum folate and vitamin B12 in various epileptic patients. Biomed Rep. 2016;5(4):413-416.

21. Xu Y, Zhang N, Xu S, Xu H, Chen S, Xia Z. Effects of phenytoin on serum levels of homocysteine, vitamin B12, folate in patients with epilepsy: a systematic review and meta-analysis (PRISMA-compliant article). Medicine (Baltimore). 2019;98(12):e14844.

22. Becit-Kizilkaya M, Oncu S, Bugra Koca H, Cavusoglu D. Evaluation of anti-seizure medications and their serum concentration with regard to cardiovascular risk parameters: a cross-sectional study. Epilepsy Behav. 2024;155:109802.

23. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2(3):299-316.

24. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. BMJ. 2014;349:5226-5231.

25. Jones KS, Collins D, Meadows SR, Koulman A, Page P. National Diet and Nutrition Survey data reveal a decline in folate status in the United Kingdom population between 2008 and 2019. Am J Clin Nutr. 2023;118(6):1182-1191.

26. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-1930. Erratum in: J Clin Endocrinol Metab. 2011;96(12):3908. Erratum in: J Clin Endocrinol Metab. 2024;109(10):e1991.

27. Seğmen F, Aydemir S, Küçük O, Dokuyucu R. The roles of vitamin D levels, gla-rich protein (GRP) and matrix gla protein (MGP), and inflammatory markers in predicting mortality in intensive care patients: a new biomarker link? Metabolites. 2024;14(11):620.

28. Julian T, Syeed R, Glascow N, Angelopoulou E, Zis P. B12 as a treatment for peripheral neuropathic pain: a systematic review. Nutrients. 2020;12(8):2221.

29. Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. Ann Neurol. 2011;69(2):352-359.

30. Reyes Fernandez PC, Wright CS, Warden SJ, Hum J, Farach-Carson MC, Thompson WR. Effects of gabapentin and pregabalin on calcium homeostasis: implications for physical rehabilitation of musculoskeletal tissues. Curr Osteoporos Rep. 2022;20(6):365-378.

31. Alles SRA, Cain SM, Snutch TP. Pregabalin as a pain therapeutic: beyond calcium channels. Front Cell Neurosci. 2020;14:83.

32. He Q, Li J. The evolution of folate supplementation - from one size for all to personalized, precision, poly-paths. J Transl Int Med. 2023;11(2):128-137.

33. Li Y, Bromley R, Meador KJ, Bjork MH, Pennell PB, Voinescu PE; Epilepsy in the Childbearing Age through Menopause Consortium (ECAM). Folate supplement dosage for pregnant people with epilepsy: survey of international experts recommendation and current practices. Epileptic Disord. 2025;27(6):1209-1216.

34. Eghrari NB, Yazji IH, Yavari B, Van Acker GM, Kim CH. Risk of dementia following gabapentin prescription in chronic low back pain patients. Reg Anesth Pain Med. 2025:rapm-2025-106577. Epub ahead of print.

35. Mohan A, Kumar R, Kumar V, Yadav M. Homocysteine, vitamin B12 and folate level: possible risk factors in the progression of chronic heart and kidney disorders. Curr Cardiol Rev. 2023;19(4):e090223213539.

36. Sirivarasai J, Shantavasinkul PC, Thitiwiwatkul M, Monsuwan W, Panpunuan P, Sritara P. Association between plasma homocysteine, folate, vitamin B12 levels, and metabolic dysfunction indices in elderly with arterial stiffness. J Clin Med. 2025;14(9):2998.

37. Qu R, Han A, Zhao Y, Qu X, Zhu Y. Characteristics of clinical manifestations and molecular genetics of inherited hyperhomocysteinemia in children and adolescents: a single center experience from China. Front Neurol. 2025;16:1588273.