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Effects of Vitamin B12 and Folic Acid Supplementation on Negative Symptoms as the Primary Outcome and Positive and Cognitive Symptoms as Secondary Outcomes in Schizophrenia: A Randomized Controlled Trial

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Abstract

Objective: Persistent negative symptoms in chronic schizophrenia often show limited responsiveness to antipsychotic therapy. The present study evaluated whether supplementation with vitamin B12 and folic acid improves negative symptoms as the primary endpoint, while also examining effects on positive symptoms and global cognitive functioning as secondary outcomes.

Method: In this randomized, double-blind, placebo-controlled trial, 88 inpatients with chronic schizophrenia receiving stable risperidone therapy were assigned to one of four groups: vitamin B12 (1 mg/day), folic acid (1 mg/day), combined vitamin B12 plus folic acid (1 mg/day each), and placebo for eight weeks. Negative symptoms were measured using the Scale for the Assessment of Negative Symptoms (SANS), positive symptoms using the Scale for the Assessment of Positive Symptoms (SAPS), and cognitive function using the Mini-Mental State Examination (MMSE). Serum concentrations of vitamin B12 and folate were assessed at baseline and after the intervention.

Results: Supplementation with vitamin B12 and/or folic acid led to significant increases in serum concentrations of the corresponding vitamins ($P < 0.001$). All supplementation groups demonstrated significant and clinically meaningful reductions in negative symptom severity compared with placebo, with large effect sizes. In contrast, no significant changes were detected in positive symptoms or global cognitive performance.

Conclusion: Adjunctive vitamin B12 and folic acid supplementation significantly reduced negative symptom severity in chronic schizophrenia, without affecting positive symptoms or global cognition. Targeted correction of vitamin deficiencies may represent a valuable adjunctive approach for persistent negative symptoms.

Key words: *Cognition; Dietary Supplements; Folic Acid; Schizophrenia; Vitamin B12*

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Chronic schizophrenia is a serious and long-standing mental health condition marked by enduring positive symptoms, including hallucinations and delusional thinking, along with negative and cognitive disturbances that significantly compromise social functioning and overall well-being. While antipsychotic drugs are typically successful in alleviating positive symptoms, negative and cognitive impairments—such as reduced motivation, blunted emotional expression, social disengagement, and deficits in executive processes—continue to show limited responsiveness to current pharmacological interventions. These enduring symptom domains are particularly prominent in individuals with a prolonged course of illness and represent major barriers to functional recovery, vocational integration, and independent living (1, 2).

Growing evidence suggests that nutritional factors, particularly deficiencies in vitamin B12 and folate, may contribute to the pathophysiology and clinical expression of schizophrenia (3-6). Vitamin B12 and folate are essential cofactors in one-carbon metabolism, a biochemical pathway critical for DNA synthesis, repair, and methylation processes that are fundamental to normal neurodevelopment and neuronal function. Emerging evidence indicates that nutritional factors, especially deficiencies in vitamin B12 and folate, may contribute to both the biological mechanisms and clinical expression of schizophrenia (3-6). These vitamins serve as essential cofactors in one-carbon metabolism, a pathway required for DNA synthesis, repair, and methylation processes fundamental to normal neurodevelopment and neuronal function. Insufficient levels may compromise nucleotide synthesis and reduce methylation capacity, potentially leading to epigenetic alterations implicated in negative and cognitive symptom persistence (7-10).

Disruptions in one-carbon metabolism are likewise correlated with increased concentrations of homocysteine, a neurotoxic byproduct associated with oxidative damage, vascular endothelial impairment, and excitatory neuron injury. Elevated homocysteine concentrations have been repeatedly observed in patients with schizophrenia and are associated with greater symptom severity and poorer clinical outcomes (11-13). Population-based research suggests that a substantial proportion of patients with schizophrenia exhibits low serum folate or vitamin B12 levels, often in the absence of overt hematological abnormalities, suggesting a covert but clinically relevant nutritional vulnerability (14, 15).

Clinical studies examining supplementation with vitamin B12 and folic acid in individuals with schizophrenia have yielded inconsistent findings. Some trials have documented reductions in negative symptoms and modest enhancements in cognitive functioning after supplementation, whereas others have not observed statistically significant therapeutic effects (16-18). Heterogeneity in study findings may reflect differences in illness stage, baseline nutritional status, genetic variability affecting folate metabolism, intervention duration, and outcome measures (7, 19). Notably, evidence suggests that disturbances in one-carbon metabolism may preferentially influence negative symptoms through effects on epigenetic regulation, inflammatory signaling, and dopaminergic hypofunction in prefrontal cortical circuits, rather than the mesolimbic dopaminergic mechanisms primarily underlying positive symptoms (20).

Despite increasing interest in nutritional interventions for schizophrenia, data from chronically ill populations in Middle Eastern countries remain limited. Dietary patterns, genetic background, and healthcare access in these regions may differ substantially from those in Western populations, potentially modifying treatment response. Moreover, many prior trials have focused on early-stage psychosis or genetically stratified samples, leaving uncertainty regarding the role of vitamin-based interventions in people with long-standing illness and stable antipsychotic treatment (3, 9, 21, 22).

Accordingly, the present double-blind, randomized, placebo-controlled trial was designed to evaluate the effects of vitamin B12 and folic acid supplementation, administered alone or in combination, on symptom domains in patients with chronic schizophrenia. We hypothesized that correction of vitamin B12 and folate status would lead to significant improvement in negative symptoms, designated as the primary outcome, while effects on positive symptoms and cognitive function were examined as secondary outcomes. By integrating clinical symptom assessment with baseline and post-intervention measurement of serum vitamin concentrations, this study aimed to clarify the clinical relevance of targeted nutritional supplementation in a chronic schizophrenia population and to inform adjunctive treatment strategies tailored to this underrepresented clinical context.

Materials and Methods

Study Design and Setting

This study was conducted as a randomized, double-blind, placebo-controlled clinical trial at a psychiatric rehabilitation center affiliated with a university hospital. The trial was designed in accordance with CONSORT guidelines. The primary objective was to evaluate the effects of vitamin B12 and folic acid supplementation, administered alone or in combination, on deficit symptoms in patients with persistent schizophrenia. Secondary objectives were to assess effects on positive symptoms, cognitive function, and serum vitamin concentrations.

This investigation was conducted as a randomized, double-blind, placebo-controlled clinical trial at a university-affiliated psychiatric rehabilitation center and adhered to CONSORT guidelines. Schizophrenia diagnoses were established according to DSM-5-TR

criteria and confirmed through structured interviews conducted by board-certified psychiatrists. Ethical approval was obtained from the institutional review board (approval code: IR.USWR.REC.1401.120), and the study was registered in a recognized clinical trial registry (registration number: IRCT20221018056233N1). All procedures complied with the Declaration of Helsinki.

Participants and Recruitment

Between December 2023 and September 2024, a total of 431 patients were screened for eligibility. Of these, 343 patients were excluded because they did not meet inclusion criteria, declined participation, or for other predefined reasons. 88 inpatients with chronic schizophrenia were ultimately enrolled using convenience sampling. The participant flow, including screening, randomization, and follow-up, is presented in the CONSORT flow diagram (figure 1).

Inclusion criteria were: age between 18 and 65 years, a diagnosis of schizophrenia for at least two years, stable risperidone treatment with a daily dose of 6 mg or less for a minimum of 12 weeks prior to enrollment, no use of vitamin B12 or folate supplements during the preceding three months, absence of current or recent substance use disorders, and the ability to comply with study procedures. Normal intellectual functioning was confirmed using Raven's Progressive Matrices.

Exclusion criteria included comorbid neurological or medical conditions known to affect cognition (such as liver disease or megaloblastic anemia), malabsorption syndromes or special dietary restrictions, pregnancy or lactation, and cognitive or clinical conditions that precluded informed consent or adherence to the study protocol.

Adjunctive biperiden (up to 6 mg/day) was permitted for the management of extrapyramidal symptoms, and clonazepam (up to 1 mg/day) was allowed as needed for insomnia under clinical supervision. The medication dosages were maintained at consistent levels for the duration of the study. Before being enrolled, all participants signed a written informed consent form.

Sample Size Estimation

The required sample size was calculated according to the primary endpoint, defined as the change in severity of negative symptoms assessed using the Scale for the Assessment of Negative Symptoms (SANS). Prior randomized controlled studies evaluating folate and vitamin B12 supplementation in individuals with schizophrenia — including a multicenter trial that demonstrated greater improvement in negative symptoms with combined folate and vitamin B12 compared with placebo, as well as other controlled investigations examining the impact of folate on negative symptom domains — have reported substantial effect sizes (Cohen's d ≈ 0.80) (22, 23). Using a two-tailed significance level of 0.05 and a statistical power of 80%, at least 22 participants were needed in each group. Considering that the study included four groups, the overall required sample size was therefore 88 participants.

Randomization and Blinding

Participants were randomly assigned in a 1:1:1:1 ratio to one of four intervention groups: folic acid (1 mg/day), vitamin B12 (1 mg/day), combined folic acid plus vitamin B12 (1 mg/day each), or placebo, for a duration of eight weeks. Randomization was performed using a computer-generated random sequence created with the RAND function in Microsoft Excel.

Allocation concealment was ensured by an independent hospital pharmacist who was not involved in participant assessment or clinical care. Active supplements and placebo tablets were identical in appearance, packaging, and labeling. Participants, treating clinicians, nursing staff, and outcome evaluators remained unaware of group assignments during the entire study period.

Interventions

Study medications were administered orally once daily for eight weeks. Adherence to supplementation was supervised by nursing staff who were not involved in outcome assessment. No changes to antipsychotic treatment were permitted during the study period unless clinically necessary.

Outcome Measures

The primary outcome measure was change in negative symptom severity from baseline to week 8, assessed using the SANS. The SANS includes 25 items assessing five distinct symptom domains and is scored on a six-point scale, where higher scores reflect more severe symptoms (24, 25).

Secondary outcome measures included positive symptom severity, assessed using the Scale for the Assessment of Positive Symptoms (SAPS), and global cognitive function, assessed using the Mini-Mental State Examination (MMSE). The SAPS includes 34 items assessing hallucinations, delusions, bizarre behavior, and formal thought disorder (25, 26).

The MMSE is a 30-point screening instrument evaluating orientation, attention, memory, language, and visuospatial abilities. Serum vitamin B12 and folate concentrations were measured at baseline and at the end of the eight-week intervention period (27, 28).

At both baseline and week 8, all clinical evaluations were carried out by trained psychiatrists with no knowledge of the assigned treatments.

Statistical Analysis

All statistical procedures were carried out using IBM SPSS Statistics (version 27). The distribution of continuous variables was evaluated for normality using the Shapiro – Wilk test, along with visual inspection of distribution plots. Continuous data are reported as mean ± standard deviation or median with interquartile range, depending on data distribution. Categorical variables are summarized as counts and percentages.

Changes within groups were examined using paired-sample t-tests for variables demonstrating normal

distribution, whereas the Wilcoxon signed-rank test was applied to variables that did not meet normality assumptions. Comparisons across groups were performed using one-way analysis of variance (ANOVA) for normally distributed outcomes and the Kruskal – Wallis test for non-normally distributed data.

When significant overall group differences were identified, post hoc pairwise analyses were conducted. Tukey's test was used following ANOVA, while Mann – Whitney U tests with Holm – Bonferroni adjustment were applied after Kruskal – Wallis testing to control for multiple comparisons. Effect sizes were estimated using Cohen's d for within-group analyses, partial eta squared (η^2) for ANOVA, and epsilon squared (ε^2) for Kruskal – Wallis tests. A two-tailed P-value of less than 0.05 was considered statistically significant. Exact P values and 95% confidence intervals are reported where applicable.

Results

Participant Flow and Baseline Characteristics

The flow of participants through the study is presented in the CONSORT flow diagram (Figure 1). As shown in Figure 1, 88 inpatients with chronic schizophrenia were randomly and equally allocated to one of four intervention groups: vitamin B12 supplementation, folic acid supplementation, combined vitamin B12 plus folic acid supplementation, or placebo (n = 22 per group). All individuals assigned to the study groups finished the eight-week intervention and were included in the intention-to-treat analysis, with no missing outcome measures. All randomized participants completed the eight-week intervention and were included in the intention-to-treat analysis. No missing outcome data were recorded.

Baseline demographic, clinical, and biochemical parameters were comparable across the four study groups, reflecting effective randomization (Table 1). The mean age ranged from 46.7 to 51.6 years, with no significant differences observed between groups (one-way ANOVA, P = 0.164). Distributions of sex, educational attainment, and illness duration were similar across groups (all P > 0.05). Additionally, baseline serum levels of vitamin B12 and folate showed no significant variation between the groups (vitamin B12: Kruskal – Wallis P = 0.074; folate: ANOVA P = 0.162). (Figure 2). Similarly, baseline scores on the SANS, SAPS, and MMSE exhibited no significant between-group differences (all P > 0.05).

After eight weeks of intervention, plasma levels of vitamin B12 and folate increased significantly in the groups receiving the corresponding supplements (Table 2). In the vitamin B12 – only group, serum vitamin B12 levels increased markedly compared with the baseline (Wilcoxon signed-rank test, P = 0.001), with a mean increase of 210.7 pg/mL. A similarly significant increase was observed in the combined supplementation group (mean increase: 177.1 pg/mL; P = 0.001). In contrast, no significant changes were observed in the folate-only or placebo groups.

Between-group comparison of vitamin B12 change scores demonstrated a significant overall effect (Kruskal – Wallis H = 21.50, P < 0.001). Post-hoc pairwise analyses with Holm – Bonferroni correction confirmed that both vitamin B12 – containing groups showed significantly greater increases than the folate-only and placebo groups (adjusted P < 0.01). The between-group effect size was large (epsilon squared = 0.28).

Serum folate levels increased significantly in both the folate-only and combined supplementation groups (paired t-tests, both P = 0.001), with mean increases of 3.37 ng/mL and 3.59 ng/mL, respectively. No significant changes were observed in the vitamin B12 – only or placebo groups. Analysis of variance revealed a significant between-group effect for folate change scores ($F(3,84) = 8.23$, P < 0.001), and post-hoc Tukey tests confirmed significantly greater increases in folate-containing groups compared with placebo (P < 0.01). The effect of folate, quantified using partial eta squared change, was 0.23, indicating a large effect.

Negative Symptoms (Primary Outcome)

Negative symptom severity, assessed using the SANS (Scale for the Assessment of Negative Symptoms) total score, improved significantly in all three supplementation groups over the eight-week intervention period (Table 3; Figure 2). Participants receiving vitamin B12 supplementation demonstrated a mean reduction of 19.36 points on the SANS, reflecting a substantial effect within the group (Cohen's d = 0.95; P = 0.001). Comparable improvements were observed in the folate-only group (mean reduction: 25.00 points; d = 0.91; P = 0.001) and in the combined supplementation group (mean reduction: 26.54 points; d = 0.80; P = 0.001).

By contrast, the placebo group showed no statistically significant change in negative symptoms over the study period (mean reduction: 3.00 points; P = 0.156), with only a small effect size (d = 0.31).

Between-group analysis of SANS change scores revealed a statistically significant overall difference (Kruskal – Wallis H = 21.83, P = 0.001), with a large effect size (epsilon squared = 0.27). Post-hoc Mann – Whitney U tests with Holm – Bonferroni adjustment demonstrated that each supplementation group differed significantly from placebo (adjusted P < 0.01). No meaningful statistical differences were found among the three active supplementation groups. Negative symptom outcomes are presented in Table 3 and visualized in Figure 3.

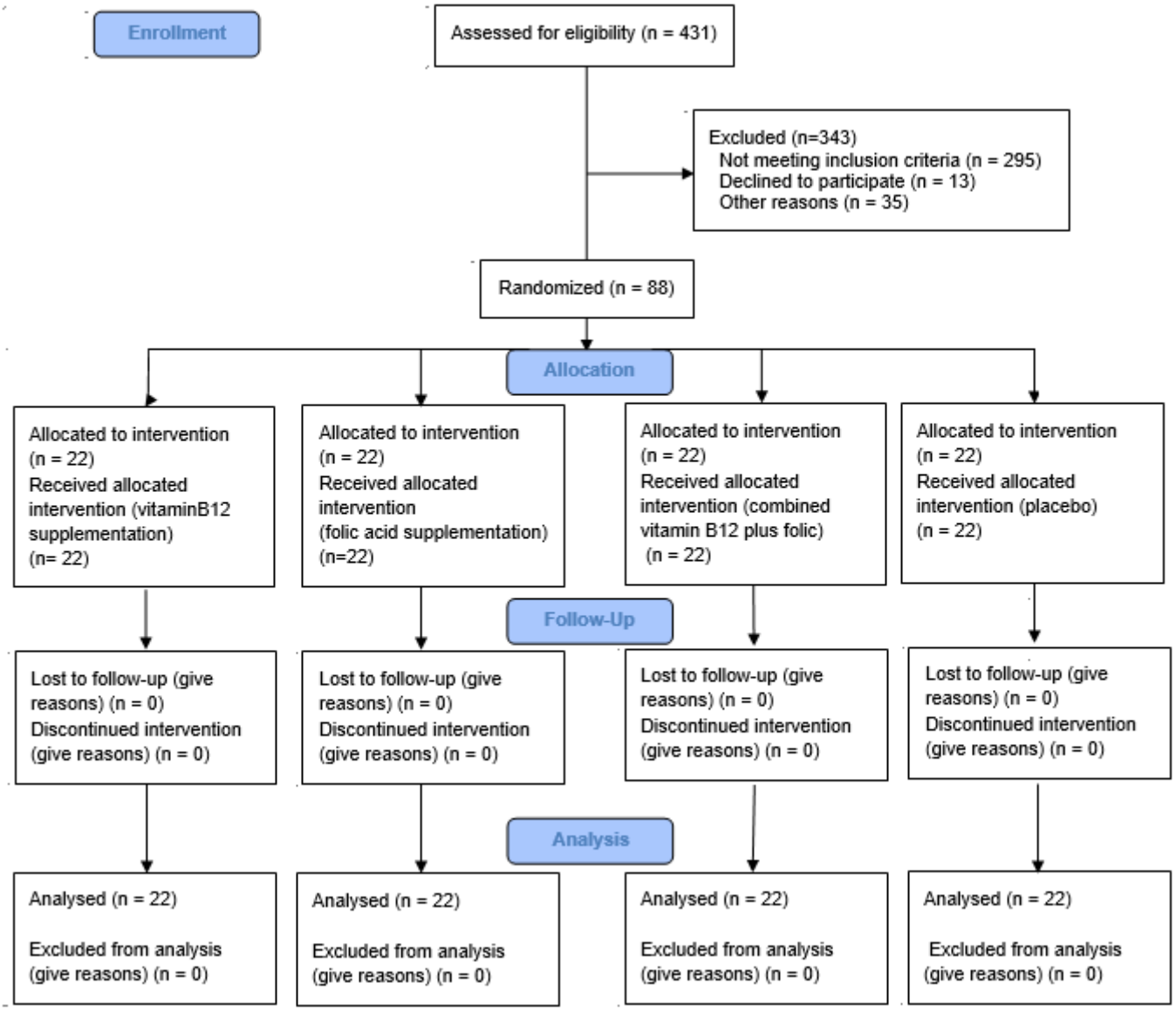

Figure 1. CONSORT Flow Diagram
Effects of Vitamin B12 and Folic Acid Supplementation on Negative, Positive and Cognitive Symptoms in Individuals with Chronic Schizophrenia

Table 1. Baseline Demographics and Clinical Characteristics of Patients with Chronic Schizophrenia in a Study of Vitamin B12 and Folate Supplementation (Mean ± SD [95% CI] or n [%])

Variable	B12 Only	Folate Only	Combination	Placebo	Test & P-value
Age (years)	49.59 ± 8.43 (45.0 – 54.2)	51.63 ± 5.26 (47.5 – 55.7)	46.72 ± 8.33 (41.4 – 52.0)	49.86 ± 6.25 (45.8 – 54.0)	ANOVA F = 1.87, P = 0.164
Male, n (%)	15 (68.2%)	15 (68.2%)	18 (81.8%)	17 (77.3%)	Fisher's exact, P = 0.705
Primary education, n (%)	6 (27.3%)	7 (31.8%)	7 (31.8%)	6 (27.3%)	Fisher's exact, P = 0.996
Serum Vitamin B12 (pg/mL)	395.81 ± 89.01 (347.9 – 443.7)	485.35 ± 137.03 (425.4 – 545.2)	464.54 ± 142.19 (403.8 – 525.3)	438.71 ± 112.46 (388.2 – 489.3)	Kruskal – Wallis H = 6.50, P = 0.074

Variable	B12 Only	Folate Only	Combination	Placebo	Test & P-value
Serum Folate (ng/mL)	7.82 ± 3.88 (5.84 – 9.80)	7.60 ± 2.57 (6.47 – 8.73)	5.88 ± 3.66 (4.10 – 7.66)	6.48 ± 2.73 (5.28 – 7.69)	ANOVA F = 1.77, P = 0.162
MMSE* score	21.59 ± 4.47 (19.3 – 23.9)	22.04 ± 4.27 (19.9 – 24.1)	19.22 ± 3.67 (17.3 – 21.1)	21.31 ± 3.99 (19.4 – 23.2)	Kruskal – Wallis H = 5.04, P = 0.135
SANS* score	78.36 ± 28.93 (65.1 – 91.6)	89.50 ± 16.43 (82.0 – 96.9)	83.22 ± 22.36 (72.5 – 94.0)	80.18 ± 24.66 (68.7 – 91.6)	Kruskal – Wallis H = 2.38, P = 0.485
SAPS score	63.77 ± 34.06 (50.1 – 77.4)	55.22 ± 27.52 (45.0 – 65.5)	52.54 ± 23.80 (43.6 – 61.4)	51.54 ± 26.98 (41.8 – 61.2)	ANOVA F = 0.83, P = 0.473

MMSE: Mini-Mental State Examination
SANS: Scale for the Assessment of Negative Symptoms
SAPS: Scale for the Assessment of Positive Symptoms

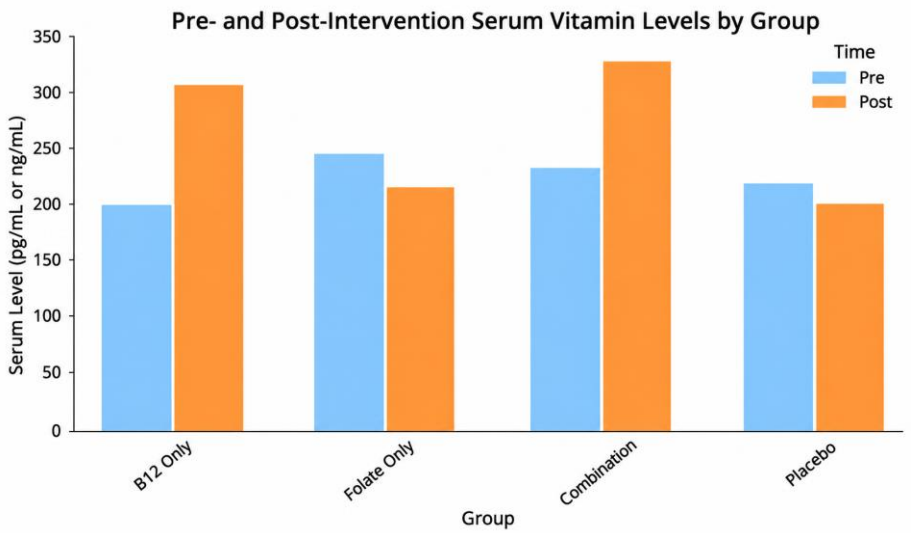

Figure 2. Serum Vitamin B12 and Folate Concentrations at Baseline and Week 8 in Patients with Chronic Schizophrenia Undergoing Vitamin B12 and/or Folic Acid Supplementation or Placebo

Table 2. Serum Vitamin B12 and Folate Levels Pre- and Post-Intervention in Patients with Chronic Schizophrenia Undergoing Vitamin B12 and/or Folic Acid Supplementation or Placebo (Mean ± SD [95% CI])

Vitamin & Group	Baseline Mean (95% CI)	Week 8 Mean (95% CI)	Change Mean ± SD (95% CI)	Within-group p-value	Between-groups P-value
Vitamin B12 (pg/mL)					
B12 Only	395.8 (347.9 – 443.7)	606.5 (525.2 – 687.7)	+210.7 ± 141.96 (147.1 - 274.1)	0.001 (Wilcoxon signed-rank test)	< 0.001 (Kruskal – Wallis test)
Combination	464.5 (403.8 – 525.3)	641.7 (569.9 – 713.5)	+177.1 ± 90.31 (137.9 - 216.3)	0.001 (Wilcoxon signed-rank test)	
Folate Only	485.4 (425.4 – 545.2)	419.8 (350.2 – 489.4)	−65.2 ± 161.19 (−122.3 - −8.0)	0.070 (Wilcoxon signed-rank test	
Placebo	438.7 (388.2 – 489.3)	400.3 (338.9 – 461.7)	−38.5 ± 101.09 (−73.6 - −3.3)	0.089 (Wilcoxon signed-rank test)	

Vitamin & Group	Baseline Mean (95% CI)	Week 8 Mean (95% CI)	Change Mean ± SD (95% CI)	Within-group p-value	Between-groups P-value
Folate (ng/mL)					
Folate Only	7.60 (6.47 – 8.73)	10.98 (9.30 – 12.66)	+3.37 ± 3.80 (2.11 - 4.64)	0.001 (paired t-test)	< 0.001 (ANOVA)
Combination	5.88 (4.10 – 7.66)	9.48 (7.90 – 11.05)	+3.59 ± 3.43 (2.53 - 4.65)	0.001 (paired t-test)	
B12 Only	7.82 (5.84 – 9.80)	7.15 (4.50 – 9.80)	−0.67 ± 2.67 (−1.62 - 0.29)	0.252 (paired t-test)	
Placebo	6.48 (5.28 – 7.69)	5.73 (4.38 – 7.07)	−0.75 ± 1.95 (−1.34 - −0.17)	0.086 (paired t-test)	

Table 3. Change in Negative Symptoms (Scale for the Assessment of Negative Symptoms) from Baseline to Week 8 in Patients with Chronic Schizophrenia Receiving Different Interventions

Group	Baseline Mean (95% CI)	Week 8 Mean (95% CI)	Change Mean ± SD (95% CI)	Within-group P-value (Wilcoxon)	Between-groups P-value (Kruskal – Wallis)
B12 Only	78.4 (65.1 – 91.6)	59.0 (42.3 – 75.7)	−19.4 ± 20.3 (−27.8 - −11.0)	0.001	
Combination	83.2 (72.5 – 94.0)	56.7 (40.7 – 72.6)	−26.5 ± 33.4 (−39.9 - −13.1)	0.001	
Folate Only	89.5 (82.0 – 96.9)	64.5 (48.2 – 80.8)	−25.0 ± 27.5 (−36.1 - −13.9)	0.001	0.001
Placebo	80.2 (68.7 – 91.6)	77.2 (66.4 – 88.0)	−3.0 ± 9.56 (−6.5 - 0.5)	0.156	

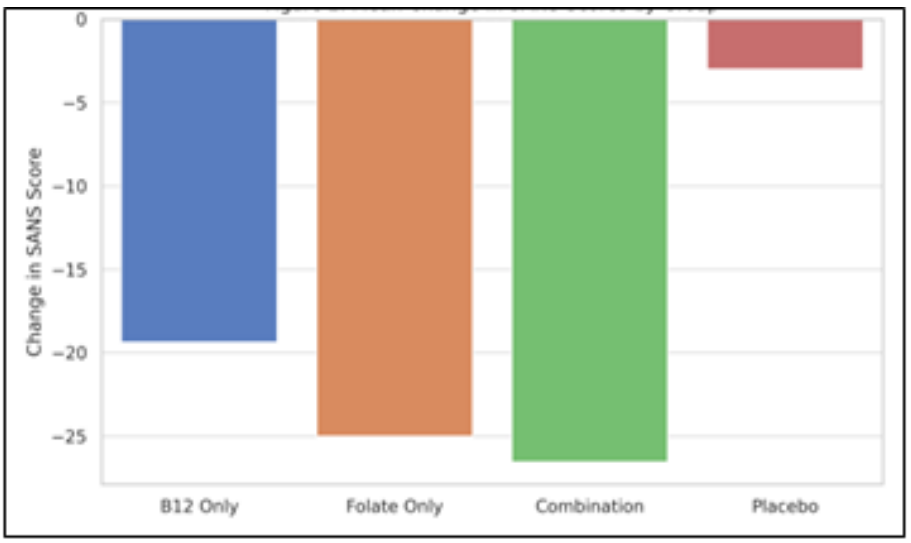

Figure3. Effect of Vitamin B12 and Folate Supplementation on Negative Symptoms (Scale for the Assessment of Negative Symptoms) in Patients with Chronic Schizophrenia

Positive Symptoms

Positive symptom severity, measured using the SAPS total score, remained stable across all four groups throughout the intervention period (Table 4). Within-group analyses revealed no statistically significant alterations in SAPS scores over the 8-week period in any group (all P > 0.05). Consistent with these findings, between-group comparison of SAPS change scores using one-way ANOVA showed no significant differences among the groups (F (3,84) = 0.13, P = 0.909), with negligible effect sizes.

Table 4. Baseline and Week 8 Positive Symptom Scores (Scale for the Assessment of Positive Symptoms) and Change Scores Across Four Treatment Groups (B12 Only, Combination, Folate Only, Placebo) in Chronic Schizophrenia

Group	Baseline Mean (95% CI)	Week 8 Mean (95% CI)	Change Mean ± SD (95% CI)	Within-group P-value	Between-groups P-value (ANOVA)
B12 Only	63.8 (50.1 – 77.4)	68.3 (53.5 – 83.1)	+4.54 ± 11.43 (−0.7 - 9.8)	0.076	
Combination	52.5 (43.6 – 61.4)	54.9 (45.6 – 64.2)	+2.31 ± 12.12 (−3.6 - 8.2)	0.380	
Folate Only	55.2 (45.0 – 65.5)	58.0 (47.4 – 68.6)	+2.81 ± 10.10 (−1.3 - 6.9)	0.205	0.909
Placebo	51.5 (41.8 – 61.2)	54.5 (43.9 – 65.1)	+2.95 ± 8.57 (0.1 - 5.8)	0.121	

Cognitive Function

Global cognitive performance, assessed using the MMSE, showed no significant variation during the study in any group (Table 5). Within-group analyses demonstrated that no statistically meaningful differences were detected in MMSE scores over time (all P > 0.05). Between-group comparison of MMSE change scores likewise revealed no significant differences (Kruskal – Wallis H = 4.63, P = 0.275). Effect sizes for MMSE changes were small (Cohen's d < 0.30), indicating no detectable impact of supplementation on global cognitive function.

Table 5. Baseline and Week 8 Mini-Mental State Examination (MMSE) Scores and Change Scores Across Four Treatment Groups in Patients with Chronic Schizophrenia

Group	Baseline Mean (95% CI)	Week 8 Mean (95% CI)	Change Mean ± SD (95% CI)	Within-group P-value (paired t)	Between-groups P-value (Kruskal – Wallis)
B12 Only	21.6 (19.3 – 23.9)	22.3 (19.9 – 24.6)	+0.68 ± 2.23 (−0.04 – 1.40)	0.179	
Combination	19.2 (17.3 – 21.1)	20.2 (18.0 – 22.4)	+0.95 ± 2.25 (0.08 – 1.82)	0.060	
Folate Only	22.0 (19.9 – 24.1)	22.4 (20.3 – 24.5)	+0.40 ± 2.50 (−0.62 – 1.42)	0.451	0.275
Placebo	21.3 (19.4 – 23.2)	20.0 (16.5 – 23.6)	−1.31 ± 3.99 (−3.16 – 0.54)	0.336	

Summary of Findings

In summary, eight weeks of intake of vitamin B12 and/or folic acid resulted in significant increases in serum vitamin concentrations and produced robust, indicating clinically meaningful improvements in negative symptoms compared with placebo. In contrast, no meaningful impacts were observed on positive symptoms or global cognitive performance.

Discussion

In this double-blind, randomized, placebo-controlled trial, adjunctive supplementation with vitamin B12 and/or folic acid resulted in significant and clinically meaningful improvements in negative symptoms among patients with chronic schizophrenia. These improvements were observed consistently across all supplementation groups and were accompanied by robust increases in serum concentrations of the

administered vitamins, confirming adequate biological exposure. In contrast, supplementation had no significant effect on positive symptoms or global cognitive performance.

The observed improvement in negative symptoms is consistent with a growing body of literature supporting the role of B vitamins as adjunctive treatments in schizophrenia, particularly for symptom domains that are poorly responsive to antipsychotic medications. Several randomized controlled trials and meta-analyses have reported beneficial effects of folate and vitamin B12 supplementation on negative symptoms, with effect sizes comparable to those observed in the present study (17, 18, 22, 29). The finding that all supplementation strategies—vitamin B12 alone, folic acid alone, and their combination—were associated with significant symptom reduction suggests that the correction of deficiencies in one-carbon metabolism may be sufficient to produce clinical benefit in this domain (10, 30-32).

The biological plausibility of these findings could be attributed to the involvement of vitamin B12 and folate in one-carbon metabolism, a critical process for DNA methylation, neurotransmitter synthesis, and regulation of inflammatory and oxidative stress pathways. Disruptions in these processes have been implicated in the pathophysiology of negative symptoms, particularly through effects on prefrontal cortical functioning and dopaminergic signaling (33, 34). Although homocysteine was not measured in the present study, the significant elevation of serum vitamin levels supports the interpretation that metabolic capacity was enhanced during the intervention period.

Notably, the combined supplementation group demonstrated the greatest numerical reduction in negative symptom severity, although differences among active treatment groups were not statistically significant. This pattern is consistent with prior work suggesting potential additive or synergistic effects of combined folate and vitamin B12 supplementation, while also indicating that either vitamin alone may confer meaningful benefit in appropriately selected patients (10, 30-32).

In contrast to the robust effects on negative symptoms, no significant changes were observed in positive symptoms or cognitive performance. These findings align with previous studies indicating that positive symptoms are largely driven by dopaminergic mechanisms that are less directly influenced by nutritional interventions (16, 18, 35, 36).

The absence of cognitive improvement may reflect several factors, including the chronicity of illness in the study population and the use of the Mini-Mental State Examination as a global cognitive screening tool, which may lack sensitivity to detect changes in specific cognitive domains relevant to schizophrenia (37-39).

Our findings differ from those reported by Chen *et al.* (2024), who did not observe significant effects of folate and vitamin B12 supplementation on either positive or negative symptoms (14).

Several methodological and clinical differences may account for this discrepancy, including variations in illness duration, baseline nutritional status, intervention length, and population characteristics. In particular, regional dietary patterns, genetic variability affecting folate metabolism, and differences in symptom severity may influence responsiveness to supplementation and underscore the importance of contextualizing nutritional interventions within specific patient populations.

The marked increases in serum vitamin B12 and folate achieved in the supplemented groups are clinically and biologically significant, given that deficiencies in these vitamins are consistently linked with greater negative symptom severity and poorer clinical outcomes (22, 40-43).

Our results align with several prior randomized controlled trials. For instance, Roffman *et al.* demonstrated alleviation of negative symptoms with folate plus vitamin B12 supplementation, particularly among genetically stratified subpopulations (22). Similarly, Levine *et al.* reported symptomatic benefits of combined B vitamins in patients with elevated homocysteine levels (44). Additionally, a systematic review highlights the promise of B vitamins in targeting deficit symptoms and cognitive impairments in schizophrenia, underscoring the relevance of our findings (16).

Taken together, the present results support the potential utility of Vitamin B12 and folate therapy as safe, accessible, and cost-effective adjunctive treatments for persistent negative symptoms in chronic schizophrenia. While supplementation should not be considered a replacement for antipsychotic therapy, routine assessment of nutritional status and targeted correction of deficiencies may represent a pragmatic strategy to address symptom domains that remain inadequately treated with standard pharmacotherapy.

Limitation

Several limitations should be considered when interpreting these findings. The relatively short intervention duration (eight weeks) and moderate sample size may have limited the detection of smaller or delayed effects, particularly for cognitive outcomes. Although serum vitamin B12 and folate concentrations were quantified to confirm biological exposure, functional markers of one-carbon metabolism (e.g., homocysteine) were not assessed, limiting mechanistic interpretation. Dietary intake of vitamin B12 and folate was not formally quantified; however, all participants were long-term inpatients receiving a standardized hospital diet, which likely reduced interindividual variability. Cognitive function was assessed using the MMSE, a global screening tool with limited sensitivity to the domain-specific cognitive deficits characteristic of schizophrenia; therefore, null cognitive findings should

be interpreted cautiously. Finally, although concomitant medications such as biperiden and clonazepam were kept at stable doses, their potential effects on negative or cognitive symptoms cannot be entirely excluded.

Conclusion

In summary, this randomized, double-blind, placebo-controlled study shows that adding vitamin B12 and/or folic acid as a supplement markedly alleviates the severity of negative symptoms in individuals with chronic schizophrenia, while having no detectable effect on positive symptoms or global cognitive function over an eight-week period. These findings provide clinical support for integrating nutritional assessment and targeted vitamin supplementation into comprehensive treatment strategies for patients with persistent negative symptoms. Subsequent studies should aim to determine which patient subgroups are most likely to respond to supplementation and on optimizing intervention duration and biomarker-guided treatment approaches.

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Conflict of Interest

None.

Author's Contributions

Conception or design:,Omid Rezaei, Mahnaz Shahali, Gita Sadighi; Data acquisition: Mahnaz Shahali, Gita Sadighi,Omid Rezaei Data analysis: Gita Sadighi , Omid Rezaei, Mahnaz Shahali; Data interpretation: Gita Sadighi ,Omid Rezaei, Mahnaz Shahali, Reza Momeni Shideh; Drafting: ,Gita Sadighi, Reza Momeni Shideh, Omid Rezaei, Mahnaz Shahali;Critical review: all authors; Final approval: all authors; All authors are accountable for all aspects of the work.

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