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ORIGINAL ARTICLE

Decreased hepatic enzymes reflect the decreased vitamin B6 levels in Parkinson's disease patients

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Abstract

The study aims to investigate the vitamin B6 levels in Parkinson's disease (PD) patients and their association with liver enzymes and evaluate how much dysregulation is associated with levodopa dose. Furthermore, to evaluate the effect of Opicapone, a catechol-o-methyl-transferase inhibitor, on vitamin B6 levels by monitoring the AST and ALT levels in patients treated with Levodopa–Carbidopa Intestinal Gel Infusion (LCIG). For these aims, serum vitamin B6 levels were measured (PD, $n = 72$ and controls, $n = 31$). The vitamin B6 level was compared with the total levodopa dose, clinical parameters, and blood homocysteine, albumin, and hemoglobin levels in PD patients. Correlations between vitamin B6 levels and AST and ALT levels, as well as the ratio ALT/AST, were analyzed. Changes in the AST and ALT levels and ALT/AST were analyzed in the patients treated with LCIG before and after the therapy ($n = 24$) and in the patients treated with LCIG + Opicapone before and after Opicapone treatment ($n = 12$). We found vitamin B6 levels were significantly lower in PD patients. Total levodopa dose and albumin levels were independently associated with vitamin B6 levels. Decreased vitamin B6 levels appeared as lower AST and ALT levels and ALT/AS. Treatment with LCIG decreased the AST and ALT levels and ALT/AST. Adjunctive therapy with Opicapone to LCIG ameliorated the decreased ALT and ALT/AST. We conclude that the ALT and ALT/AST can be useful parameters for monitoring vitamin B6 levels and Opicapone can ameliorate the dysregulated vitamin B6 in PD patients.

KEYWORDS
biomarker, COMT inhibitor, Levodopa-Carbidopa Intestinal Gel Infusion, Opicapone, Parkinson's disease, vitamin B6

Abbreviations: 3-OMD, 3-O-methyldopa; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COMT, catechol-o-methyl-transferase; LCIG, Levodopa–Carbidopa Intestinal Gel Infusion; PD, Parkinson disease; PLP, Pyridoxal-5-phosphate.

1 | INTRODUCTION

Levodopa treatment has been the most effective therapy for more than a half-century for the treatment of the motor symptoms of Parkinson's disease (PD). Recently, a significant improvement in the levodopa delivery has been achieved with the use of extended-release-tablets or through the continuous delivery of levodopa/carbidopa using Levodopa–Carbidopa Intestinal Gel Infusion (LCIG), which accelerates the utilization of levodopa.[1–3] Moreover, the development of drugs that support the effect of levodopa, such as monoamine oxidase B inhibitors and catechol-o-methyl transferase (COMT) inhibitors, also supports the trend.

Although the continuous drug delivery of levodopa is effective for the treatment of motor fluctuations in advanced PD patients, some of the metabolic changes produced by the levodopa metabolism are known to generate adverse events in PD patients, such as polyneuropathy caused by decreased levels of vitamin B6.[4–7] For example, high-dose levodopa treatment can cause vitamin B6 deficiency when levodopa is metabolized to 3-o-methyldopa (3-OMD) by COMT in the peripheral tissue and enhances the methionine cycle. In this process, homocysteine (Hcy) is produced, and vitamin B6 is consumed to metabolize Hcy. Theoretically, COMT inhibitors can be useful in preventing the cascade of increasing Hcy level and vitamin B6 consumption; however, to the best of our knowledge, a few studies have evaluated the efficacy of COMT inhibitors in ameliorating the dysregulated metabolism produced caused by high-dose levodopa therapies, except there is one study conducting the 1-day application of the COMT inhibitors and showed the beneficial effects on the metabolic changes by levodopa treatment, such as high blood Hcy.[8]

In this study, in order to retrospectively analyze the change in dysregulated methionine cycle-related molecules induced by a high-dose levodopa treatment, we analyzed the correlation between the total levodopa dose, vitamin B6 levels, and serum AST and ALT levels in PD patients. The AST and ALT levels, routinely measured in laboratory follow-ups, are also indicators of the vitamin B6 status. Pyridoxal-5-phosphate (PLP), one of the forms of vitamin B6, is a required cofactor of several enzymes, including AST and ALT. Thus, a decrease in vitamin B6 will reduce the activities of both AST and ALT. However, a reduction in PLP affects more severely the activity of ALT than AST. In consequence, lower levels of vitamin B6 will produce lower values in the ratio ALT/AST.[9] By analyzing the change in AST and ALT levels before and after the LCIG and Opicapone treatments, we indirectly investigate how the high-dose levodopa treatment affected the vitamin B6 levels and how the Opicapone treatment ameliorated the dysregulation of vitamin B6 and related methionine cycle.

2 | MATERIALS AND METHODS

2.1 | Participants

In this study, patients who agreed to participate in our cohort study of PD and the Osaka University Biomarker Study for Neuromuscular Diseases were recruited. All the recruited patients were diagnosed with clinically established PD according to the Movement Disorder Society Diagnostic Criteria for PD.[10] Patients who received vitamin B therapies were excluded. First, the vitamin B6 levels and the total levodopa dose, AST, ALT, and homocysteine levels in 72 PD patients were compared. Second, changes in AST and ALT levels were analyzed before and after the initiation of LCIG treatment in 24 patients receiving LCIG treatment. The characteristics of the LCIG treated group are listed in Table S2. Finally, the changes in AST, ALT, and homocysteine levels were analyzed before and after the treatment with Opicapone in 12 patients as adjunctive therapy with LCIG. The characteristics of the Opicapone treated group are listed in Table S2. The disease controls included 31 patients with Stroke ($n=11$), polyneuropathy ($n=4$), multiple sclerosis ($n=4$), amyotrophic lateral sclerosis ($n=3$), multiple system atrophy ($n=2$), myasthenia gravis ($n=2$), and other disorders ($n=5$).

2.2 | Measurement of vitamin B6 levels

Serum vitamin B6 levels were determined by high-performance liquid chromatography by the company SRL. Inc (Tokyo, Japan), following the protocol previously reported by Yoshida et al.[11]

2.3 | Statistical analyses

Pearson's correlation analysis was performed to analyze the correlations between parameters. We considered $p<.05$ as statistically significant and correlation coefficients (r)$>.4$ as sufficient. Multivariate regression analyses with stepwise variable selection ($\alpha=.05$ for inclusion and $\alpha=.10$ for exclusion) was performed to evaluate the effect of clinical parameters and laboratory data on the vitamin B6 level. Statistical Package for the Social Sciences 23.0J software (IBM Japan, Tokyo, Japan) was used for statistical analysis.

2.4 | Bias

Our data sources were patients who agreed to participate in a cohort study of PD and the Osaka University Biomarker Study for Neuromuscular Diseases.

3 | RESULTS

3.1 | Serum vitamin B6 levels decreased in PD patients and correlated with the levodopa dose

When levodopa is metabolized by COMT, homocysteine is produced by the methionine cycle, and vitamin B6 is consumed to metabolize homocysteine; therefore, vitamin B6 levels tend to decrease in PD patients treated with levodopa. We first confirmed that the vitamin B6 levels were significantly lower in PD than in disease controls

(Figure 1A, $p = .0153$; the information of the participants is summarized in Table S1).

We next performed multivariate linear regression analysis and demonstrated that among age, sex, disease duration, disease severity, total levodopa dose, blood hemoglobin, homocysteine, and albumin, the total levodopa dose and the albumin levels were independent factors that were associated with the vitamin B6 levels (Table 1). We included blood albumin, hemoglobin, and homocysteine because these factors are known to affect the vitamin B6 levels.[12,13]

We confirmed that the vitamin B6 levels strongly correlated with the total levodopa dose (Figure 1B, $r = .483$, $p < .0001$) but weakly with the albumin levels (Figure 1C, $r = .372$, $p = .004$).

3.2 | Serum vitamin B6 levels are correlated with AST, ALT levels, and the ratio ALT/AST

PLP is a cofactor of several enzymes, including AST and ALT[9]; then, their enzymatic activities will decrease if there is a deficiency of vitamin B6. Moreover, many of the hospital laboratory examinations of AST and ALT levels are based on measuring their enzymatic activity; therefore, a reduction in the apparent levels of AST and ALT would reflect a vitamin B6 deficiency. First, we confirmed that serum AST and ALT levels were significantly decreased in PD patients compared with disease controls (AST, Figure 2A, $p = .005$, ALT, Figure 2B, $p < .0001$). Interestingly, the decrease in ALT was more significant than in AST, and the ALT/AST ratio was significantly lower in the PD group (Figure 2C, $p < .0001$). This change is reasonable because the enzymatic activity of ALT is more sensitive to the decrease of PLP than AST.[9] We, then, compared the correlation of AST, ALT levels, and AST/ALT with vitamin B6 levels (Figure 2D–F; red circles, PD; blue circles, control). We found that the decline in vitamin B6 levels was strongly associated with the decline in the AST, ALT levels, and AST/ALT in PD patients. These results provide supporting evidence for monitoring vitamin B6 levels of PD patients, especially by ALT and ALT/AST.

3.3 | LCIG treatment leads to an increased dose of levodopa and decreased AST and ALT levels

We, then, investigated how the introduction of LCIG alters AST, ALT levels, and ALT/AST before and after the LCIG treatment initiation. The characteristics of the LCIG-treated group are shown in Table S2. In general, patients who started LCIG treatment could be treated with fewer medications or even only with levodopa monotherapy[14]; therefore, LCIG treatment leads to an increase in the levodopa dose. Indeed, in our cases, the levodopa dose significantly increased (Figure 3A, $p < .0001$), while the AST and ALT levels significantly decreased (Figure 3B, $p = .031$ and 3C $p < .0001$, AST and ALT, respectively). Moreover, the ratio ALT/AST significantly diminished (Figure 3D, $p = .0002$). We did not observe a significant correlation between the duration of LCIG administration and the change in the liver enzyme levels before and after the LCIG treatment by Pearson's correlation analysis (AST; $r = 0.272$, $p = .100$, ALT; $r = 0.200$, $p = .175$, and ALT/AST; $p = .065$, $r = 0.381$).

3.4 | Opicapone treatment reduced the levodopa dose and ameliorated the decreased AST and ALT levels and the hyperhomocysteinemia in LCIG-treated patients

Then, we retrospectively analyzed the laboratory data of 12 patients who started Opicapone treatment as adjunctive therapy for LCIG. The characteristics of the LCIG + Opicapone group are shown in Table S2. First, the total levodopa dose was significantly decreased after Opicapone treatment (Figure 4A, $p = .001$). Moreover, Opicapone treatment ameliorated the dysregulated ALT level and the ratio ALT/

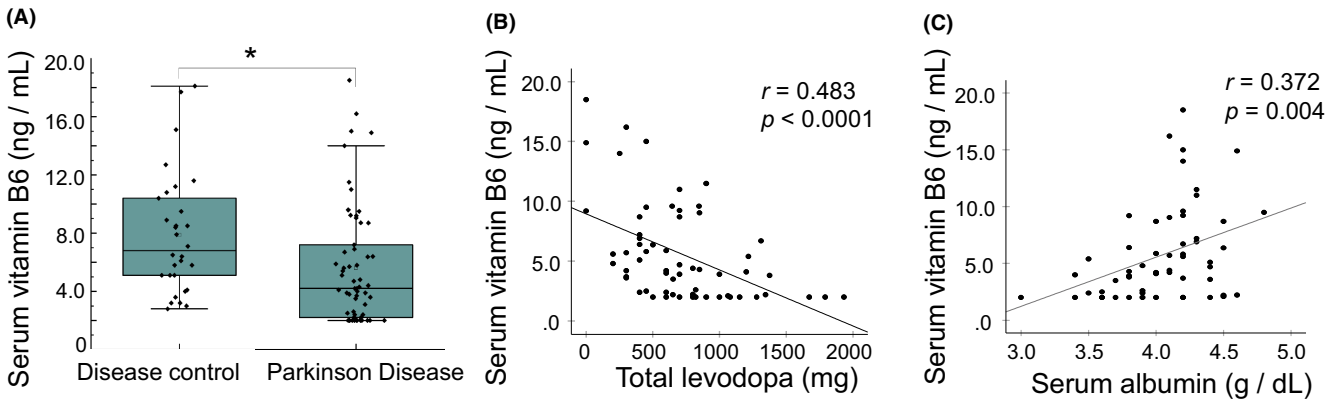


FIGURE 1 Serum vitamin B6 levels were decreased and related to the total levodopa dose in PD patients. (A) Serum vitamin B6 levels of disease control patients ($n = 31$) and PD patients ($n = 72$). (B) Correlation between the serum vitamin B6 levels and the total levodopa dose ($n = 72$). (C) Correlation between the serum vitamin B6 levels and the serum albumin levels ($n = 72$). *$p < .05$, Student's t-test (A). Pearson's correlation analysis was used for evaluating the statistical analysis (B, C). PD, Parkinson's disease.

AST, but not AST (AST, Figure 4B, $p=.201$, ALT, Figure 4C, $p=.0086$, and ALT/AST, Figure 4D, $p=.024$). Interestingly, the correlation between total levodopa and serum ALT level before and after Opicapone treatment indicates that Opicapone ameliorated the ALT decrease more strongly than the mere effect on the decrease of levodopa dose (Figure 4E). We also analyzed the correlation between the duration of Opicapone administration and the change in the liver enzymes before and after the treatment, and we did not find any significant correlations between them (AST; $r=-0.18$, $p=.100$, ALT; $r=0.143$, $p=.329$, and ALT/AST; $p=.266$, $r=0.202$). Finally, we checked the change in the homocysteine levels in blood by the Opicapone treatment. We found that Opicapone significantly ameliorated the high homocysteine concentration in blood (Figure 4F, $p=.037$).

TABLE 1 Multivariate linear regression analysis with stepwise variable selection; comparison among serum vitamin B6 and clinical and laboratory data ($n=72$).

	Vitamin B6	
	Coefficient (95% CI)	*p*-Value
Total levodopa	−0.004 (−0.007 to −0.003)	0.001
Serum albumin	3.186 (0.327 to 6.045)	0.030
Age	n.a.	0.233
Sex	n.a.	0.544
Disease duration	n.a.	0.862
Hoehn–Yahr scale	n.a.	0.157
Serum hemoglobin	n.a.	0.560
Plasma homocysteine	n.a.	0.076

4 | DISCUSSION

In this study, we showed that the adjunctive therapy with Opicapone was effective against vitamin B6 deficiency, that occurs in patients on high-dose levodopa receiving LCIG treatment. Since this is a retrospective study and vitamin B6 was not measured longitudinally, we first showed that AST and ALT levels and vitamin B6 are strongly correlated in PD patients and that the ratio ALT/AST is lower when vitamin B6 is low, indicating its significance as a biomarker for monitoring vitamin B6 in PD patients. The correlation was not significant in control groups, probably because many factors other than vitamin B6 may impact liver enzyme levels in the control group. Then, we showed that ALT levels and the

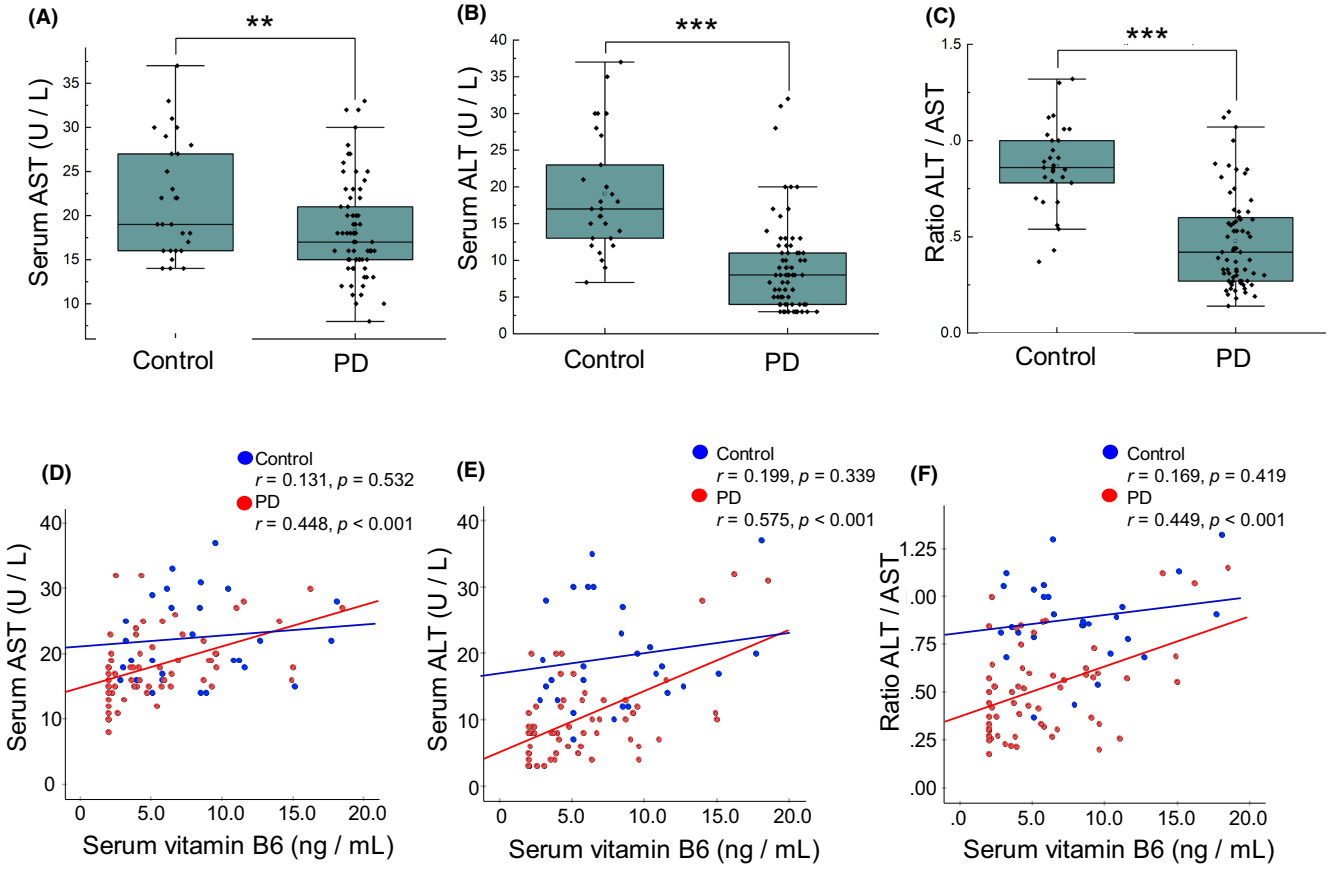
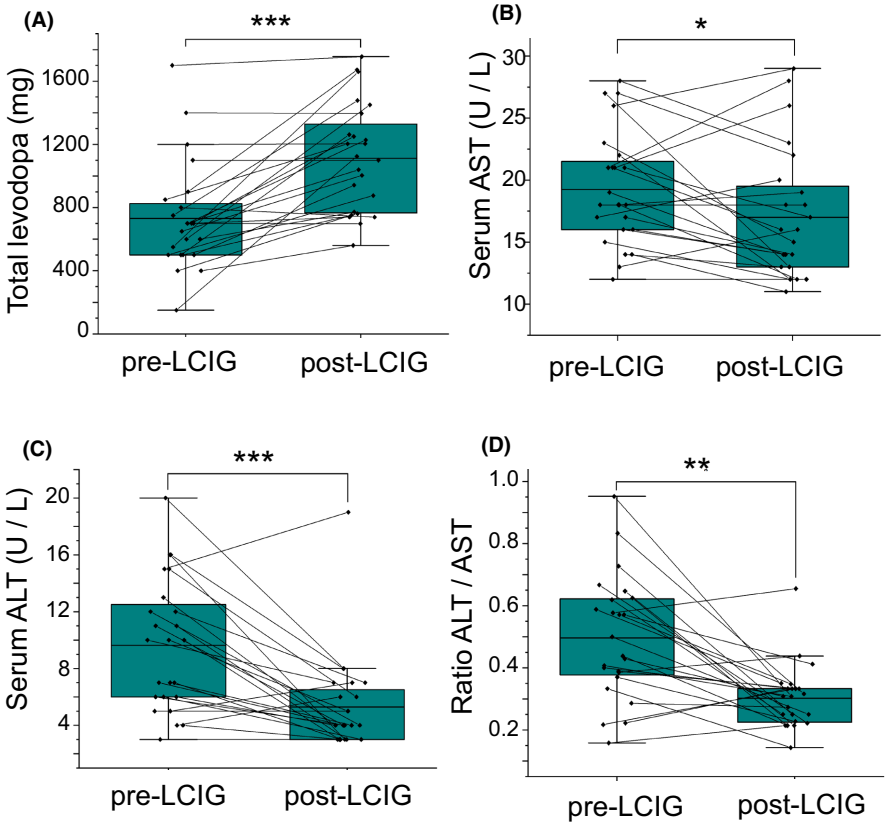


FIGURE 2 Comparison between the serum vitamin B6 levels and the serum AST and ALT levels, and the ratio ALT/AST. (A–C) Comparison between controls and PD of AST (A), ALT (B), and the ratio ALT/AST (C). (D–F) Correlation between the serum vitamin B6 levels and the AST level (D), ALT level (E), and the ratio ALT/AST (F) ($n=72$). Red circles and line, PD patients ($n=72$) and blue circles and line, controls ($n=31$). Student's *t*-test (A–C). $^{*}p<.05$; $^{**}p<.001$; $^{***}p<.0001$. PD, Parkinson's disease.



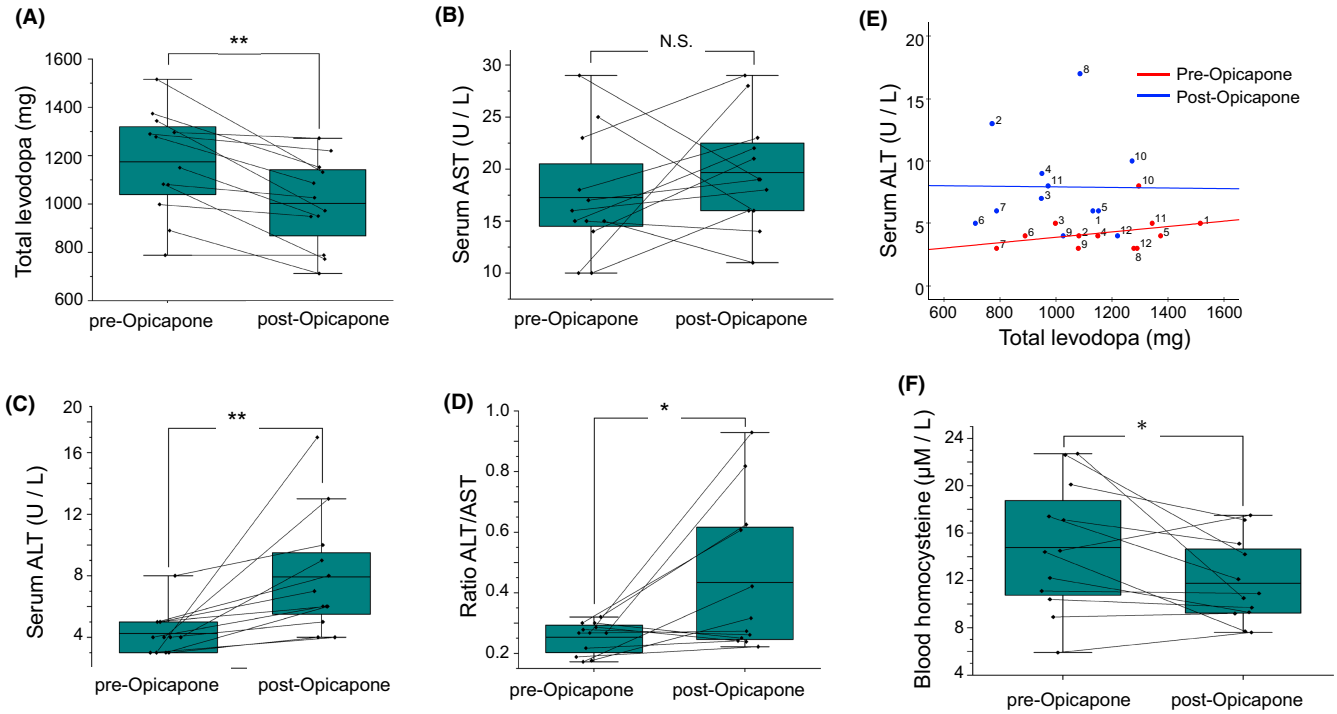


FIGURE 3 Changes in the levodopa dose and the blood AST and ALT levels after LCIG treatment. (A–D) Comparison between pre- and post-LCIG treatment of total levodopa dose (A), AST level (B), ALT level (C), and the ratio ALT/AST (D). $n = 24$. $^{*}p < .05$; $^{**}p < .001$; $^{***}p < 0.0001$. Student's *t*-test. LCIG, Levodopa–Carbidopa Intestinal Gel Infusion.

FIGURE 4 Changes in the levodopa dose and the blood AST and ALT levels, the ratio ALT/AST, and the homocysteine levels after Opicapone treatment. (A–D) Comparison between pre- and post- Opicapone treatment of total levodopa dose (A), AST level (B), ALT level (C), the ratio ALT/AST (D), and homocysteine (F). (E) Correlation between total levodopa dose and ALT level. Red and blue dots and the line represents pre- and post-Opicapone groups, respectively. $n = 12$ (A–F). $^{*}p < .05$; $^{**}p < .001$; $^{***}p < .0001$. Student's *t*-test (A–D, F). Pearson's correlation analysis (E).

ratio ALT/AST improved with the use of Opicapone. Although this is an indirect proof of concept, this is the first report that demonstrates an improvement in the vitamin B6 deficiency associated with high-dose levodopa by using Opicapone. Interestingly, the substantial improvement in ALT with Opicapone, considered here as an improvement in vitamin B6, suggests that the effect was not only a reduction of levodopa dose, but also the inhibition of the methionine cycle through the inhibition of COMT. We also observed a decrease in the blood homocysteine levels following Opicapone use, confirming the effect of the improved methionine cycle. Interestingly, we did not observe any significant correlation between the changes in liver enzyme levels before and after the LCIG administration or Opicapone administration and the duration of those treatments. We checked the level of liver enzymes in 24 days for LCIG and 63.5 days for Opicapone after administration; therefore, the vitamin B6 change appeared as the liver enzyme change might happened earlier than those durations.

It should be noted that there are some limitations in this study. One is that this study is a retrospective study. Second, most of the participants were treated with levodopa/carbidopa, and a few patients were treated with levodopa/benserazide, therefore, we could not compare the different effects between the decarboxylase inhibitors. Third, future studies will be required to confirm if the vitamin B6 decreases with levodopa and improves with Opicapone in prospective ways. Finally, the vitamin B6 change was only indirectly validated by ALT and the ratio ALT/AST.

In this study, vitamin B6 levels were measured cross-sectionally in 72 PD patients, the largest number to our knowledge. In addition, multivariate analysis has been performed to examine factors that affect vitamin B6 levels within PD patients. While the correlation of vitamin B6 levels with the total levodopa dose was primary, a weak correlation with albumin was also observed, identifying it as an independent factor in the present study. Since albumin is a stabilizer of vitamin B6 in the blood, we consider the albumin reduction is an independent factor of vitamin B6 reduction. In addition, patients with PD commonly suffer from gastrointestinal dysfunction as a non-motor complication, so we speculate that several patients develop hypoalbuminemia due to malabsorption. Here, we confirmed that blood albumin levels are negatively correlated with the Hoehn–Yahr stage ($r = -.501$, $p < .001$, graph not shown), indicating that hypoalbuminemia in advanced Parkinson disease may further accelerate the vitamin B6 depletion.

Finally, we showed the possibility of improvement of decreased levels of vitamin B6 by Opicapone treatment. We believe that this study will provide important insights in the levodopa-centered therapy for advanced PD.

AUTHOR CONTRIBUTIONS

KI, YK, ST, TA, KK, and HM designed the experiments. JD, CA, and NW collected and stored the blood sample. KI performed the statistical analysis. KI, YK, CA, CC, ST, JD, NW, TA, KO, KK, YK, and HM discussed the results and wrote the manuscript. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors report no sources of funding and no conflicts of interest.

DATA AVAILABILITY STATEMENT

All the raw data used in this study will be deposited in the UMIN Individual Case Data Repository, and all those data are publicly available to other researchers. Further details of the data-sharing policy are available at http://www.umin.ac.jp/icdr/index.html. All the data related to the results are available from the corresponding authors.

ETHICS STATEMENT

This study was conducted as part of our cohort study of PD and the Osaka University Biomarker Study for Neuromuscular Diseases. This study was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects endorsed by the Japanese government. The Ethics Committee of Osaka University Graduate School of Medicine approved this study (approval numbers: 13471 and 19089).

PATIENT CONSENT STATEMENT

All patients were informed about the study and provided written consent. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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