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Evaluation of hyperhomocysteinemia in the progression of Parkinson's disease

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Abstract:

Objective*:* To assess the prevalence of hyperhomocysteinemia in Parkinson's Disease (PD) and if this elevated serum level can be used as a predictive biomarker in risk assessment for the progression of cognitive decline in PD.

Design**:** Systematic Literature Review

Methods: A literature search was conducted through Google Scholar and Pubmed using phrases such as, "Parkinson's Disease", and "homocysteine", "Parkinson's progression. Three articles, measuring serum homocysteine levels and cognitive functioning in PD patients, were selected, analyzed, and compared to assess for a relationship between homocysteine levels and cognitive decline in PD.

Results: Elevated homocysteine levels are associated with worse cognitive scores in some areas of neurocognitive testing in PD patients as compared to those with normal homocysteine levels.

Conclusion: Though study results do show an inversely proportional relationship between homocysteine serum levels and cognitive function, the presence of confounding variables within PD subjects, as well as, the unknown physiologic mechanism of homocysteine and neurons, makes it difficult to find a causal relationship between elevated homocysteine and neurocognitive function in PD.

Introduction:

Parkinson's disease (PD), the second most prevalent neurodegenerative condition after Alzheimer's disease, is characterized by a progressive loss of motor and cognitive function[1]. The precise mechanism of neurodegenerative decline is largely unknown and there are few laboratory tests to track its progress. The diagnosis and progression of PD is based upon its distinctive clinical features from the history and neurological exam. Though it is understood that a deficiency in dopamine and atrophy of the substantia nigra is involved in the disease process, these pathological pathways are difficult to track until it is too late or without extensive imaging[1]. Thus, it would be in the interest of the medical community to find an easy

laboratory finding, such as a serum level, to track the progression of PD before symptoms manifest.

Recent research has suggested that homocysteine, though the mechanism in PD is not wholly understood, may be a reliable predictor of cognitive decline. Homocysteine is a non-protein sulfur containing amino acid that is naturally occurring via metabolism of methionine, an essential amino acid[1]. Elevated serum homocysteine levels, hyperhomocysteinemia, can be caused by deficiencies in folate and vitamin B6 or B12 because of their direct effect in the biochemical pathway of homocysteine. Hyperhomocysteinemia has also been observed in patients with renal failure, atherosclerotic and thrombotic vascular disease[2]. Most notably, high levels of serum homocysteine, due to its possible neurotoxic effects, has been linked to Alzheimer's disease. Homocysteine has agonistic effects on the glutamate binding site of the NMDA receptor, stimulating the receptors, causing an excitotoxic response[2]. Homocysteine disrupts the DNA methylation cycle inducing DNA damage, cell death, and alterations in gene expression, which can trigger apoptosis and neuronal dysfunction[2]. Though it is not well understood, hyperhomocysteinemia's neurotoxic effects may be linked to the cognitive decline seen in PD.

Historically, Levodopa (L-dopa) is associated with causing hyperhomocysteinemia. The suggestion of using hyperhomocysteinemia as a biomarker predicting cognitive decline could alter the recommendations for treating PD with L-dopa[3]. The goal of this research is to analyze for an in situ relationship between homocysteine serum levels and cognitive decline in PD, independent of L-dopa. The three research articles test serum homocysteine levels and cognitive decline in patients with PD to assess for an association. The implications of this relationship could shape the clinical course and treatment of PD.

PICO:

P (Population) → Patients with diagnosis of Parkinson's Disease (PD)

I (Intervention) → Elevated serum homocysteine levels

C (Comparison) → Lack of current biomarkers

O (Outcomes) → Predicting cognitive decline in PD

Clinical Question:

Can hyperhomocysteinemia be linked as a causative agent in the progression of cognitive decline in PD?

Methods:

A systematic literature search was conducted using Google Scholar and PubMed for articles that reviewed the relationship between serum homocysteine levels and cognitive decline in PD (see Figure 1). The following keywords were used: Parkinson's disease, homocysteine, Parkinson's progression, and homocysteine levels. Two investigators reviewed the articles independently and uncertainties were resolved through discussion. Initial screening criteria excluded articles that were in non-English, non-human, prior to 2016, meta-analyses, review articles, or full-text articles not available.

Eligibility criteria for study inclusion consisted of: 1) studies evaluating the possible relationship between homocysteine and cognitive decline in PD patients. Studies that had no relevant outcomes or had non-Parkinson's controls were excluded; 2) studies evaluating PD that used medications as a variable for stratification. Many studies have shown that L-dopa can increase levels of homocysteine, so this would be a confounding variable if stratified; 3) studies with sufficient results with serum homocysteine and neurocognitive testing.

The quality of the studies were assessed by the two investigators based on the sample size, homocysteine measurement, and cognitive measurement. The final articles were chosen based on their similarities and the inclusion/exclusion criteria listed above. No statistical analysis has been done thus far.

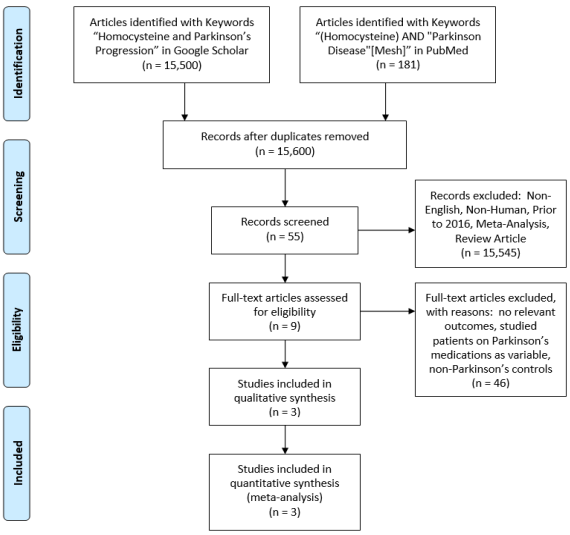

Figure 1: PRISMA flow diagram depicting the literature review resulting in the three studies evaluated.

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Results:

Study Comparison			
Title & Author	Homocysteine and Cognitive Function in Parkinson's Disease, Licking et al.[4]	Vitamin B12 and Homocysteine Levels Predict Different Outcomes in Early Parkinson's Disease, Christine et al.[5]	Elevated Serum Homocysteine Levels Have Differential Gender-Specific Associations with Motor and Cognitive States in Parkinson's Disease, Bakeberg et al.[6]
Year Published	2017	2018	2019
Sample Size	294	680 (456 follow up)	205
Journal	*Parkinsonism & Related Disorders*	*Movement Disorders*	*Parkinson's Disease*
Cognitive Measurement	Neurocognitive testing of memory, visuospatial, executive functioning, and language	UPDRS, ambulatory capacity score (sum of UPDRS items 13-15, 29&30), and MMSE, calculated as annualized rates of change	MDS-UPDRS III and H&Y scale (physical), Clinical psychologist and Neuropsychological assessments, and Addenbrooke's cognitive function
Homocysteine Measurement	Homocysteine chromatography, Serum	Fasting total homocysteine (tHcy), Serum	Fasting total homocysteine (tHcy), Serum collected prior to clinical or psychological assessments
Elevated Homocysteine Level Cut off	14 □mol/L	15 □mol/L	N/A
Outcomes	Elevated homocysteine was linked with verbal memory function and semantic fluency memory, but an overall causal relationship between homocysteine and cognitive function was unclear.	Elevated homocysteine predicted greater cognitive decline with decreased MMSE scores.	Elevated serum homocysteine levels are associated with a greater motor impairment in males with Parkinson's disease and poorer cognitive performance in females with Parkinson's disease.

Table 1: Table comparing the demographics of three articles[4,5,6] analyzed in this review.

Study One: Homocysteine and Cognitive Function in Parkinson's Disease, Licking, et al.[4]

Objective: This 2017 study investigated the hypothesis that elevated homocysteine levels are related to a decline in cognitive function in those with Parkinson's Disease.

Study Design: 294 patients diagnosed with idiopathic PD, some on treatment and some without, diagnosed via the United Kingdom Parkinson's Society Brain Bank (see Table 2), from the Pacific Northwest Udall Center (PANUC) were given a battery of neurocognitive testing and had their homocysteine levels measured [4,7]. Neurocognitive testing included tests for memory, executive function, language, and visuospatial function[4]. After this testing, a diagnosis was given via consensus conference, either as "no impairment", "mild cognitive impairment", or "dementia", based on the Movement Disorder Society's recommendations[4]. The consensus conference included neuropsychologists and neurologists that evaluated the patients holistically, including their testing scores, previous history, and Clinical Dementia Rating. There were several follow-ups with neurocognitive testing and serum testing from 2010 to 2012.

With each visit, plasma was collected via phlebotomy and frozen until testing. The plasma was tested via chromatography to assess for homocysteine levels. Elevated levels of homocysteine were considered if the levels were greater than 14 □mol/L[4].

The study used least squares, a form of regression analysis to assess the relationship between cognitive function and hyperhomocysteinemia. Confounding variables, such as age, gender, Hoehn & Yahr stage, MDS-UPDRS-III score, years of education, and the presence of APOE4 allele, were corrected to try to control a fraction of the variation between subjects[4].

Inclusion Criteria	Exclusion Criteria
Patients with the Pacific Northwest Udall Center that were diagnosed with idiopathic PD based on United Kingdom's Parkinson's Disease Society Brain Bank (UKBB) criteria[7].	Failure to meet UKBB criteria for idiopathic PD or history of neurological disorder than could affect cognition, like large vessel stroke or severe traumatic brain injury.

Table 2: Inclusion and exclusion criteria of subjects used in Licking, et al.

Study Result: The overall result of the study was that elevated plasma homocysteine is related to some elements of cognitive decline, but has an overall unclear effect on cognition. Initially, it was found that hyperhomocysteinemia was associated with many aspects of cognitive decline (as seen in table 2). However, once confounding variables were corrected for, such as age and gender, the association was diminished, thus, making the relationship unclear.

After correction for confounding variables, hyperhomocysteinemia was still inversely related to verbal memory function and semantic verbal fluency. Verbal memory function included delayed recall and immediate recall. These results were statistically significant after mediation with adjusted p values of less than .004, and .049, respectively[4]. It was also noted that with each follow-up a trend was seen where elevated homocysteine contributed to decrease in scores for the aforementioned neurocognitive subjects, immediate recall, delayed recall, and semantic verbal fluency. There was statistical significance in these findings with p values of .012, ,009, and .004, respectively[4].

	All subjects (N = 294)	Normal homocysteine (N = 207)	Elevated homocysteine (N = 87)	Naive comparison (Uncorrected)	Covariate corrected comparison*
Digit Symbol Test	38.4 (12.02)	39.9 (12)	34.6 (11.28)	t = 3.53 (p = 0.001)	p = 0.031
Semantic verbal fluency (animals)	18.1 (6.34)	18.9 (6.36)	16.2 (5.89)	t = 3.51 (p = 0.001)	p = 0.067
Semantic verbal fluency (vegetables)	12.4 (4.58)	13 (4.51)	10.9 (4.4)	t = 3.67 (p=<0.001)	p = 0.049
Phonemic verbal fluency (FAS)	38.2 (12.63)	39.2 (12.89)	35.9 (11.74)	t = 2.06 (p = 0.041)	NS
Hopkins Verbal Learning - Immediate	21.1 (6.36)	22 (6.1)	18.6 (6.4)	t = 3.81 (p=<0.001)	NS
Hopkins Verbal Learning - Delayed	6.8 (3.77)	7.4 (3.43)	5 (4.08)	t = 4.33 (p=<0.001)	p = 0.004
Judgment of Line Orientation	11.8 (2.42)	11.9 (2.42)	11.6 (2.42)	t = 1.01 (p = 0.313)	NS
Letter-Number Sequence Test	9.8 (3.1)	9.9 (3.07)	9.4 (3.18)	t = 1.08 (p = 0.283)	NS
MMSE	27.6 (2.17)	27.7 (2.17)	27.4 (2.16)	t = 1.11 (p = 0.27)	NS
MOCA	24.3 (3.52)	24.3 (3.42)	24.2 (3.76)	t = 0.09 (p = 0.929)	NS
Trails B (sec)	142 (87.17)	136.1 (88.73)	156.4 (81.98)	t = −1.87 (p = 0.064)	NS

Values are mean (SD).
*Corrected p-values taken from covariate corrected models with Holm-Sidak correction for multiple comparisons.

Table 3. Cognitive outcomes in normal versus elevated homocysteine from the results of Licking, et al.[4]

Critique: Positives of this study include a larger sample size of 294 with fairly specific inclusion criteria (see table 4). This study was well rounded in that it corrected for several confounding variables, giving validity to its results. A weakness lies in that there was no way to truly prove a causal relationship, as there are believed to be several other factors that lead to cognitive decline in PD. Another weakness of this study is that the study did not have exclusion criteria for treatment. Treatments, like L-dopa, are notoriously known for altering homocysteine levels, as well as improving the cognition of those with PD. Thus, medications are a huge confounding variable within this study.

Study Two: Vitamin B12 and Homocysteine Levels Predict Different Outcomes in Early Parkinson's Disease. Christine, et al.[5]

Objective: The objective of this large study was to determine the prevalence of low B12 levels and its metabolites, such as high homocysteine, high methylmalonic acid, and low holotranscobalamin, in Parkinson's disease, and if those levels coincided with clinical progression.

Study Design: 680 baseline and 456 follow-up untreated PD patients from a large study called DATATOP, Deprenyl and Tocopherol Antioxidative Therapy Of Parkinsonism, were tested for an association between B12, and its metabolites, and clinical progression of PD. DATATOP was a double-blind randomized control trial performed from 1987 to 1998 where untreated PD subjects (see table 4) received one of the four of the following treatments: deprenyl (10 mg/d) and α-tocopherol-placebo; α-tocopherol (2,000 IU/D) and deprenyl-placebo; active deprenyl and active α-tocopherol; or double placebo[5]. Baseline Serum B12, homocysteine levels, methylmalonic acid, and holotranscobalamin were collected through phlebotomy, transfused, and then frozen. These B12 levels were repeated every 3 months from 9-24 months if they subjects remained enrolled. More than 15 □mol/L was considered moderately elevated in this study.

Every 3 months, up to 24 months, PD patients were assessed for clinical progression through both physical and mental testing, including a baseline value. The cognitive function was assessed via the mini mental status exam (MMSE), Symbol digit modalities test, Selective Reminding test, and New Dot test. The cognitive decline was measured as a percent in response to their scores on the their baseline cognitive testing. The physical progression, though less important to this review, was measured via UPDRS, the unified Parkinson's Disease Rating Scale.

Inclusion Criteria	Exclusion Criteria
Subjects diagnosed with early PD without treatment.	Subjects on treatment for PD, severe PD, tremor, or diagnosed dementia (MMSE score less than or equal to 22).

Table 4: Inclusion and exclusion criteria of subjects used in Christine, et al.

Study Result: The overall results of this study, in regards to homocysteine, reported that worse baseline MMSE were seen with elevated baseline homocysteine, as well as worsening MMSE to follow-up in those with an elevated baseline homocysteine. 7% of the subjects had an elevated homocysteine at baseline and those subjects had an average decline in MMSE exam of 1.96 points (see figure 2) . Subjects without that baseline elevation saw an increase in MMSE by 0.06 points (see figure 2). These results were statistically significant with a p value of .003 and .001, respectively[5]. The researchers felt strongly that an elevated baseline homocysteine level was a predictor for worsening cognitive decline, rather than an elevation overtime. However, homocysteine levels decreased throughout the study due to treatment, so the relationship is unclear.

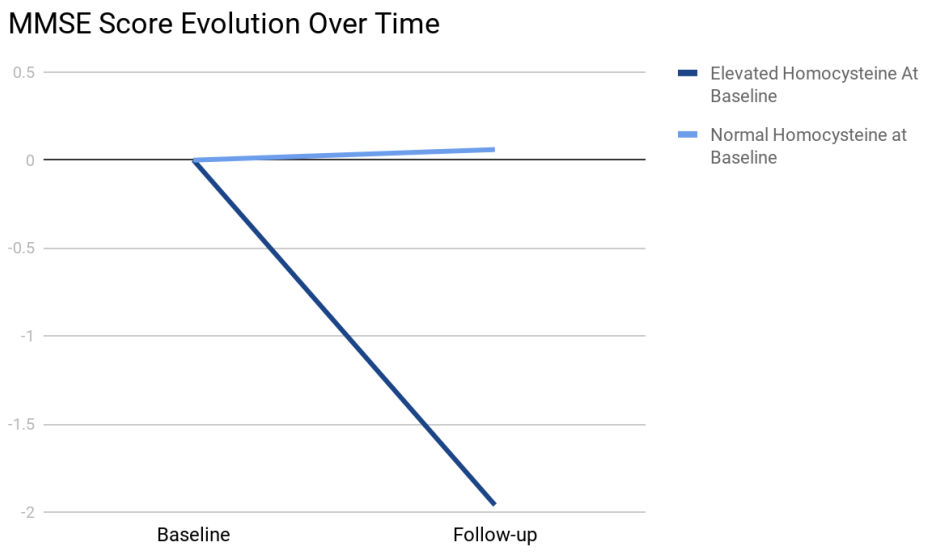

Figure 2: Line graph depicting a comparison in the change in Mini Mental Status Exam (MSSE) score from baseline to follow-up in those with elevated and normal homocysteine serum levels at baseline from Christine, et al.

Critique. There are several positives within this study. One, is the large sample size at 680 baseline and 456 follow-up subjects. This large sample size gives the study more validity. The research study was very specific in including only patients without treatment initially and correcting for various confounding variables during statistical analysis, such as age and gender.

There are also several negatives in this study. This research was sponsored by the

Michael J. Fox Foundation, potentially introducing some bias to the study as it is a heavily publicized foundation. Also, there is no explanation as to why there were 224 patients loss to follow-up. If the patients lost to follow-up were at a worse disease state, this could introduce bias into the results by skewing them towards a less severe result.

Study Three: Elevated Serum Homocysteine Levels Have Differential Gender-Specific Associations with Motor and Cognitive States in Parkinson's Disease, Bakeberg et al.[6]

Objective: This study aimed to investigate elevated serum homocysteine levels, with serum folate and vitamin B12 levels as controls, and its association with motor and cognitive symptom progression in a cohort of PD patients, with the consideration of gender-specific differences.

Study Design: 205 home-based patients with Parkinson's Disease (128 males and 77 females) and 78 (31 males and 47 females) aged-matched healthy controls were sequentially recruited from Movement Disorders Clinics Perth, Australia, Melbourne, Victoria, and Sydney, New South Wales, between 2012 and 2015 (refer to table 5). All PD patients were diagnosed in accordance with the UK Brain Bank criteria for idiopathic PD.

All PD medications were converted to a total levodopa equivalent dose (LED). Motor symptoms were evaluated in the "ON" state using the MDS-UPDRS and H&Y Scale. In addition, each participant was evaluated by a clinical psychologist and completed a panel of neuropsychological assessments using the revised Addenbrooke's Cognitive Examination (ACE-R).

Fasted patient and healthy volunteer control blood samples were collected prior to clinical or psychological assessments, from the medial cubital vein and stored in a standard vacutainer. Serum homocysteine, vitamin B12, and folate were recorded for analysis in this study.

Data were analysed using IBM-SPSS. Where appropriate, univariate regression analysis or Mann–Whitney U-test was performed to identify differences between patient and control serum markers. Generalised linear models (GLM) were used to investigate outcome measures,

such as motor severity and cognitive score, with serum Hcy as an independent variable. Variables included in the GLMs were as follows: age at assessment, LED, DBS history, and disease duration. A significant nominal p value of ≤ 0.05 was employed.

Inclusion Criteria	Exclusion Criteria
Home-based patients diagnosed with idiopathic PD based on UKBB criteria, recruited from movement disorder clinic from 2012 to 2015.	Failure to meet UKBB criteria for idiopathic PD, which was conducted by a movement disorder neurologist.

Table 5: Inclusion and exclusion criteria of subjects used in Bakeberg, et al.

Study Result: Mean comparisons revealed significant differences in serum homocysteine in males and females with PD. In male patients, a significant positive correlation between homocysteine levels and MDS-UPDRS III ($r_s = 0.319$, $p < 0.001$) was observed. In contrast, there was no significant association between homocysteine levels and MDS-UPDRS III scores in females. Conversely, in female patients, homocysteine levels were significantly inversely correlated with cognitive scores, as indicated by the ACE-R total score ($r_s = -0.449$, $p < 0.001$), whereas there was no association between homocysteine levels and cognitive scores in male PD patients.

Critique: There are many positives within this study, such as having controls to compare normal homocysteine levels and the gender-specific differences. This study also included patients without treatment of Levodopa and correlated their symptoms with levodopa equivalent doses. Although home-based PD patients were recruited to overcome selection bias, the study did not include patients with more advanced PD who were no longer independent. This was a cross-sectional study, so the levels of homocysteine over the course of the disease were not measured. Further, as the ACE-R does not include tests of executive function.

Discussion:

Parkinson's disease (PD) is a chronic and progressive neurological disease that is characterised by the onset of an array of motor and nonmotor signs and symptoms. The motor symptoms include resting tremor, rigidity, akinesia, bradykinesia, and postural instability, and the non-motor symptoms include cognitive impairment, apathy, emotional disturbance, and sleep disturbance[3]. Little is known about the specific pathological mechanism behind the death of neurons in PD, so factors that can predict these varying outcomes and potential diagnostic biomarkers of this degenerative disease require exploration.

There are three main hypotheses that are being explored in this paper: 1) Homocysteine is an indirect neurotoxin that can cause some of the neurocognitive decline in PD; 2) If the first hypothesis is true and there is a causative effect between hyperhomocysteinemia and PD, then decreasing the levels of serum homocysteine during the treatment of PD can decrease some of the cognitive decline; 3) If the first statement is false and there is no causative effect between hyperhomocysteinemia and PD, then an alternative reason must be considered and serum homocysteine can possibly be used as a biomarker to predict the progression of cognitive decline or even the rate of decline in PD.

There are many theories of how homocysteine may be neurotoxic. For one, there is some evidence that homocysteine has an agonistic relationship the glutamate binding site on the NMDA receptor, causing excitotoxic reactions within neurons[1]. Also, studies show that elevated homocysteine levels are associated with an increase in the dopaminergic neurotoxin, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)[1]. Lastly, there is evidence that homocysteine increases cellular oxidative stress[1]. Several in vitro studies have found this toxicity occurs in various types of neurons, including cortical, postnatal cerebellar granule, cerebellar Purkinje, dorsal root ganglions, hippocampal, and trigeminal sensory[1]. The studies that were analyzed in this paper were able to demonstrate correlations between hyperhomocysteinemia and cognitive decline in PD but fall short of demonstrating a causal relationship.

Several studies researching the potential relationship between serum homocysteine and PD progression exist. The three studies analyzed in this paper include two main variables in

their research: one, neurocognitive testing and two, serum homocysteine collected the day of mental status testing [4,5,6]. These papers also included discussions that statistically analyzed the relationship between serum homocysteine and cognitive function[4,5,6]. Two, Bakeberg, et al., and Christine, et al., also included data and analysis on serum homocysteine levels and the progression of physical symptoms in PD[5,6]. Bakeberg et al, Christine,et al., and Licking, et al, found inversely proportional relationships between serum homocysteine levels and some aspects of cognition in specific populations in PD patients. However, there are many confounding variables, such as age, gender, and dementia, that can influence the levels of serum homocysteine, making it difficult to analyze as a variable in PD. In this research, an overarching theme seen was that patients with hyperhomocysteinemia were generally older with a longer duration of disease. These two variables, age and length of disease, are known to contribute to the cognitive decline sequelae seen in PD. Thus, it is difficult to isolate homocysteine in these studies as the cause of the cognitive decline, when these subjects also have many risk factors for cognitive decline in PD.

The authors of the third study believe that their findings of a gender effect may help to explain previous discrepancies in the literature surrounding the utility of homocysteine as a biomarker in PD[6]. They also controlled for the confounding variable of age by comparing their serum homocysteine levels to age-matched healthy controls. This demonstrates that research is heading in the right direction and it warrants further consideration in future larger studies of PD. These future studies may tease out how serum homocysteine can be helpful in the treatment of PD.

Conclusion:

If futures studies were able to better support the theory of the inverse relationship between homocysteine and cognitive decline in PD, treatment and monitoring of the disease could be changed and potentially improved. The implications from this potential relationship are of the utmost importance. For one, L-dopa, a mainstay pharmacologic treatment for PD, is known to elevate homocysteine levels[3]. If the inverse relationship is true, L-dopa could be unknowingly increasing cognitive decline in PD patients. This could propagate a shift in

pharmacologic treatment for PD management. Also, with the current state of PD monitoring, patients are left with an unpredictable prognosis and unknown disease status. A more concrete serum level, like homocysteine, would be advantageous in allowing patients and their caretakers to prepare for cognitive decline and potentially begin preventative treatment earlier.

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