

# Serum B12, Homocysteine Levels, and their Effect on Peripheral Neuropathy in Parkinson's Disease: Indian Cohort

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## Abstract

**Background:** Cobalamin deficiency, either due to dietary inadequacy or increased consumption attributable to levodopa-mediated metabolic disturbance, and resultant hyperhomocysteinemia may contribute to peripheral neuropathy (PN) in Parkinson's disease (PD). **Aim:** The aim of the study is to assess the prevalence of Vitamin B12 deficiency, hyperhomocysteinemia in Indian PD patients, and their association with PN. **Materials and Methods:** Clinical details were collected in 93 patients over a period of 2 years. Seventy controls were included in the study. Serum B12, homocysteine, folate, electroneurography, and autonomic function tests were done. The prevalence of B12 deficiency and hyperhomocysteinemia in PD patients and controls was assessed. The association of B12 and homocysteine levels with patients' age, disease duration, levodopa equivalent daily dose, cumulative levodopa dose, Unified Parkinson's Disease Rating Scale-III off score, modified Hoehn and Yahr score, and presence or absence of PN was studied. **Results:** Serum B12, homocysteine levels, prevalence of B12 deficiency, and hyperhomocysteinemia were no different between cases and controls. Seven of 93 (9.68%) PD patients had PN. The median values of serum B12, folate, and homocysteine levels across patients with or without PN could not be compared as only seven of our patients had PN. **Conclusion:** The prevalence of B12 deficiency, hyperhomocysteinemia, and incidence of PN among our patients is very less when compared to the Western population. The conjecture that PN in PD patients may be secondary to B12 deficiency/hyperhomocysteinemia stands as a speculation.

**Keywords:** B12, homocysteine, Indian Parkinson's disease, peripheral neuropathy

## INTRODUCTION

Idiopathic Parkinson's disease (PD) is the second most common neurodegenerative disease with prevalence of approximately 360/1 lakh population and an incidence of 18/1 lakh/year.<sup>[1]</sup>

Peripheral neuropathy (PN) has been reported in more than a third of PD patients in many Western studies.<sup>[2,3]</sup> The pathophysiology of PN in PD is still unclear. Demonstration of alpha-synuclein in the dermis and unmyelinated fibers suggests a possible direct role of the disease itself.<sup>[4,5]</sup> There is more evidence that exposure to levodopa may be the main risk factor for the development of large-fiber axonal neuropathy.<sup>[6,7]</sup> Cobalamin deficiency, either due to inadequate diet or increased consumption due to iatrogenic levodopa-mediated cobalamin metabolic disturbance, as well as hyperhomocysteinemia in PD may be contributory.<sup>[8]</sup>

Higher serum homocysteine levels and lower Vitamin B12 levels have been noted in healthy Indians compared to the Western population, probably due to their dietary habits<sup>[9-11]</sup> rendering Indian PD patients more prone to PN.

Our study aims at assessing the prevalence of Vitamin B12 deficiency, hyperhomocysteinemia in Indian PD patients, and their association with PN. Identification of the magnitude of these two risk factors for PN could help in formulating strategies for prevention, early detection of patients at risk, and improving patient care.

## MATERIALS AND METHODS

### Patients and controls

The data pertaining to 93 PD patients who presented to Nizam's Institute of Medical Sciences (a tertiary referral hospital in Hyderabad, Telangana, India) with PD from September 2012 to September 2014 were collected and evaluated. The protocol used for this study was approved by the Institutional Review Board of Nizam's Institute of Medical Sciences, Hyderabad. All patients were diagnosed to be PD as per the United Kingdom Parkinson's Disease Brain Bank criteria by movement disorder specialists.<sup>[12]</sup>

Seventy healthy controls were selected from attendees of patients and healthy volunteers. All patients and controls, who had history of other systemic illnesses including diabetes mellitus, renal insufficiency and thyroid disorders, drugs, or toxin exposures (which are known to cause PN), those with

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family history of PN, chronic alcoholics, strict vegetarians, and people on any vitamin supplementation were excluded from the study.

## Methods

Demographic and clinical details including symptoms and signs of PN were collected from patients and controls.

### Treatment details in patients

Daily dosage of levodopa, pramipexole, ropinirole, rasagiline, entacapone, trihexyphenidyl and amantadine along with duration of exposure to each drug was collected. Levodopa equivalent daily dose was calculated for each patient.<sup>[13]</sup>

### Motor function assessment (in patients)

The motor subscale (part III) of the Unified Parkinson's Disease Rating Scale (UPDRS III)<sup>[14]</sup> in the "off" (12 h without dopaminergic medication) and "on" state (after taking 1.5 times the routine dose of levodopa – minimum of 200 mg of levodopa) as well as modified Hoehn and Yahr score (H and Y score) in "off" state<sup>[15]</sup> was performed in all PD patients to assess the motor severity of PD. Part IV section A of UPDRS scale was used to identify the presence of dyskinesias and off dystonia.

The following investigations were performed.

### Laboratory

- Serum Vitamin B12 and homocysteine levels were assessed in patients and controls, and serum folate levels were assessed in patients using chemiluminescence immunoassay by an automated machine in our laboratory
- Absolute serum Vitamin B12 deficiency was considered when levels were <200 pg/ml. Hyperhomocysteinemia was defined as levels >20 µmol/l in patients aged >60 years and levels >15 µmol/l in those <60 years. Folate deficiency was considered when levels were <4 ng/ml. These were based on normative data at our center for all the above tests.

### Electrophysiological

Electrophysiological examination (nerve conduction studies [NCSs]) was conducted under optimal conditions by a blinded clinical neurophysiologist in patients and controls.

Medtronic Keypoint machine (Denmark) was used for NCSs using surface electrodes with >45-min acclimatization (at room temperature 22°C–24°C and skin temperature >34°C). Motor conduction studies included sampling of median, ulnar, common peroneal, and posterior tibial nerves. Sensory conduction studies of median, ulnar, and sural nerves were done. Additional nerves were sampled depending on the clinical scenario.

The cutoff values of the various parameters studied were based on the normative data noted in our center. Only those who presented with combination of neuropathic symptoms (paraesthesias or loss of sensations) and signs (poor joint position and vibration sense, absent ankle reflexes, ascending

loss of pinprick, light touch, and temperature sensation, and distal limb atrophy) and with at least an abnormal parameter in one of the sampled nerves were considered as patients with PN. In case of discrepancy between clinical and electrophysiological evidence for PN, diagnosis was left to the discretion of neuromuscular specialist.

### Statistical analysis

Serum Vitamin B12 levels were considered as primary outcome parameter. Serum homocysteine levels were considered as other outcome variables. The presence or absence of PD was the primary explanatory variable. Among the patients with PD, duration of the disease, levodopa equivalent daily dose, cumulative levodopa dose, UPDRS off score, and modified H and Y score were considered as primary explanatory variables. Sociodemographic factors such as age, gender, and other disease and treatment-related variables were considered as other explanatory variables.

Descriptive analysis was carried out by mean and standard deviation for quantitative variables and frequency and proportion for categorical variables. Baseline parameters were compared between controls and PD patients using mean and standard deviation for normally distributed quantitative variables, median and interquartile range (IQR) for nonnormally distributed quantitative variables, and frequency and proportion for categorical variables. Statistical significance of these associations was tested by independent sample *t*-test, Mann–Whitney U-test, and Chi-square test.

The association between quantitative explanatory and outcome variables was assessed by calculating Pearson correlation coefficient. Univariate linear regression analysis was performed. The association between quantitative explanatory variables and ordinal variables was assessed by Spearman's rank correlation. The regression coefficients and their 95% confidence interval were presented.

IBM SPSS Statistics for Windows, Version 22.0, IBM Corp Armonk, NY, 2013 was used for statistical analysis, and *P* < 0.05 was considered statistically significant.

## RESULTS

Ninety-three patients with PD and 70 healthy controls were included in the final analysis. The mean age was slightly higher in cases, as compared to controls. Cases had higher male-to-female ratio. No statistically significant differences were observed between cases and controls in median serum Vitamin B12 (598.14 ± 471.960 pg/ml vs. 593.01 ± 498.80 pg/ml) and homocysteine levels (16.7 mg/dl, IQR: 12–22.75 vs. 16 mg/dl, IQR: 12–30.35). The prevalence of Vitamin B12 deficiency and hyperhomocysteinemia was not significantly different between cases and controls [Table 1]. The mean folate level in PD patients was 13.38 ± 8.76 ng/ml.

The mean duration of disease in our PD patients was 7.39 ± 5.12 years. The mean UPDRS "off" and "on" scores were 54.96 ± 14.13 and 15.99 ± 6.71, respectively. The mean

levodopa equivalent daily dose was  $510.89 \pm 377.05$  mg. The mean cumulative levodopa dose was  $774.95 \pm 753.86$  g. Only three patients (3%) were on catechol-O-methyltransferase (COMT) inhibitors. The mean H and Y score was  $2.65 \pm 0.63$ . Dyskinesias were seen in 42 (45.16%), peak dose dyskinesias in 39, biphasic dyskinesia in 2, and off dystonia in 1.

Among patients with PD, the factors which showed weak but statistically significant negative correlation with Vitamin B12 levels were duration of the disease (PCC =  $-0.37$ ,  $P < 0.001$ ), UPDRS-III off score (PCC =  $-0.319$ ,  $P = 0.002$ ), modified H and Y score (PCC =  $-0.245$ ,  $P = 0.018$ ), and cumulative levodopa dose (PCC =  $-0.273$ ,  $P = 0.012$ ) [Table 2].

On assessing the impact of various factors on serum homocysteine levels, only duration of disease showed a statistically significant weak positive correlation (PCC =  $-0.187$ ,  $P = 0.037$ ) [Table 3].

Seven of 93 (9.68%) PD patients had PN (symmetrical sensory axonal and symmetrical sensorimotor axonal in 3 and 4, respectively) [Table 4].

PD patients with PN were older (mean = 62.86 years as compared to 57.4 in PD without PN) and had a longer duration of the disease (median of 8 years as compared to 6 in PD without PN). Among the seven PD patients with PN, 2 (28.6%) patients with PN had Vitamin B12 deficiency in contrast to 7 out of 86 PD patients who did not have neuropathy (8%) [Table 4].

## DISCUSSION

It is still unclear whether PD is associated with changes in Vitamin B12 and folate levels. While some studies have shown reduced B12 and folate levels in patients with PD,<sup>[16]</sup> others have not shown any association.<sup>[17]</sup> In our study, we did not find any significant difference in mean serum B12 levels and prevalence of B12 deficiency between patients and controls.

**Table 1: Comparison of cases and controls**

Parameter	Cases (n=93)	Controls (n=70)	P
Age (mean±SD)	57.60±9.014	47.91±14.71	<0.001
Male: female ratio	2.32:1	1.47:1	0.14
Serum Vitamin B12 (pg/ml), median (IQR)	449 (269-717.50)	426 (265-721.50)	0.762
Serum homocysteine (mg/dl), median (IQR)	16.7 (12-22.75)	16 (12-30.35)	0.675
Prevalence of Vitamin B12 deficiency (%)	9.68	4.29	0.19
Prevalence of hyperhomocysteinemia (%)	40.86	40	0.91
Prevalence of PN (%)	7.53	4.29	0.39

SD=Standard deviation, IQR=Interquartile range, PN=Peripheral neuropathy

**Table 2: Correlation between various explanatory variables and serum Vitamin B12 levels of Parkinson's disease patients (n=93)**

Parameter	PCC	P	Regression coefficient	95% CI		P
				Lower	Higher	
Age	0.167	0.110	8.741	-2.007	19.489	0.110
Duration of disease	-0.370	<0.001	-34.137	-51.976	-16.298	<0.001
Levodopa equivalent daily dose	-0.141	0.089	-0.175	-0.432	0.081	0.178
UPDRS off	-0.319	0.002	-10.649	-17.242	-4.056	0.002
Modified H and Y score	-0.245	0.018	-184.450	-336.127	-32.773	0.018
Cumulative levodopa dose	-0.273	0.012	-0.160	-0.283	-0.036	0.012

PCC=Pearson's correlation coefficient, CI=Confidence interval, UPDRS=Unified Parkinson's Disease Rating Scale, H and Y=Hoehn and Yahr score

**Table 3: Correlation between various explanatory variables and serum homocysteine levels of Parkinson's disease patients (n=93)**

Parameter	PCC	P	Regression coefficient	95% CI		P
				Lower	Higher	
Age	0.061	0.279	0.072	-0.171	0.314	0.559
Duration of disease	0.187	0.037	0.384	-0.037	0.805	0.073
levodopa equivalent daily dose	0.012	0.455	0.000	-0.005	0.006	0.910
UPDRS off	0.165	0.057	0.123	-0.030	0.276	0.114
Modified H and Y score	0.092	0.191	1.535	-1.942	5.011	0.383
Cumulative levodopa dose	0.139	0.104	0.002	-0.001	0.005	0.207

PCC=Pearson's correlation coefficient, CI=Confidence interval, UPDRS=Unified Parkinson's Disease Rating Scale, H and Y=Hoehn and Yahr score

**Table 4: Profile of Parkinson's disease patients with peripheral neuropathy**

	Age (years)	Sex	Disease duration (years)	Cumulative levodopa dose (g)	Levodopa equivalent daily dose (mg)	Serum Vitamin B12 (pg/ml)	Serum homocysteine (mg/dl)	Serum folate (ng/ml)	Type of PN
Patient 1	63	Male	10	282.5	275	101	34.2	9.1	Symmetric sensory axonal neuropathy
Patient 2	67	Female	14	766.5	200	281	24	4.6	Symmetric sensory motor axonal neuropathy
Patient 3	57	Female	6	766.5	837.5	291	12.8	4.6	Symmetric sensory motor axonal neuropathy
Patient 4	62	Male	8	657	453	203	6.3	5.3	Symmetric sensory motor axonal neuropathy
Patient 5	68	Male	8	949	675	216	12.1	11	Symmetric sensory motor axonal neuropathy
Patient 6	54	Male	10	1076.75	862.5	341	7.6	12	Symmetric sensory axonal neuropathy
Patient 7	69	Male	0.5	Treatment naive	Treatment naive	126	18.2	20	Symmetric sensory axonal neuropathy

PN=Peripheral neuropathy

Levodopa along with carbidopa is the main drug used for the treatment of PD.<sup>[18]</sup> Levodopa is methylated in both brain and peripheral tissues by the enzyme COMT using S-adenosylmethionine as the methyl donor and produces the demethylated product S-adenosylhomocysteine (SAH).<sup>[8,19]</sup> SAH is subsequently hydrolyzed to form homocysteine.<sup>[8]</sup> Homocysteine is metabolized back to methionine either by 5,10-methylenetetrahydrofolate reductase (MTHFR) or betaine-homocysteine S-methyltransferase.<sup>[20]</sup> MTHFR requires folate and Vitamin B12 as cofactors.<sup>[20]</sup> Thus, with increasing exposure of PD patients to levodopa, there will be increased consumption of Vitamin B12 and possible B12 deficiency.

Studies have demonstrated a strong negative correlation of cumulative levodopa exposure, duration, and severity of PD with Vitamin B12 levels in PD patients.<sup>[2,3,6,20]</sup> In our study, a similar negative correlation of B12 levels with PD disease severity and levodopa exposure was established, i.e., the more severe the disease (as determined by UPDRS off and modified H and Y scores) and larger the exposure to levodopa (as determined by duration of disease and cumulative levodopa dose), lesser the serum B12 levels. There was no correlation with levodopa equivalent daily dose re-emphasizing the fact that only levodopa predisposes to Vitamin B12 consumption and the use of other dopaminergic drugs has no impact on B12 metabolism.

Homocysteine is predominantly generated as a metabolite during methylation of methionine and can cause oxidative stress by various mechanisms.<sup>[21,22]</sup> There is conflicting evidence regarding the role of hyperhomocysteinemia in the pathogenesis of PD. In few *in vitro* studies, elevated homocysteine has shown to block D2 dopamine receptors and increase the vulnerability of dopaminergic neurons to various toxins causing degeneration.<sup>[23,24]</sup> However, studies on drug-naive PD patients have not shown a consistent association with homocysteine levels.<sup>[25]</sup>

It has been shown that exposure to levodopa resulted in higher serum homocysteine levels compared to levodopa-naive PD patients, possibly secondary to methylation of levodopa by COMT.<sup>[26]</sup> The use of COMT inhibitors has been shown to prevent the increase in serum homocysteine levels in PD patients on levodopa in some studies,<sup>[27,28]</sup> while others have not shown a similar beneficial effect.<sup>[29]</sup>

Homocysteine generated is remethylated by the MTHFR gene with Vitamin B12 and folate as cofactors or transulfurated by cystathionine-beta synthase with Vitamin B6 as cofactor. Apart from folate, Vitamin B12, and Vitamin B6 deficiency, polymorphisms involving the MTHFR gene C677T (especially TT homozygote) also cause hyperhomocysteinemia by reducing the enzyme activity and increasing the enzyme thermolability.<sup>[30,31]</sup>

In our study, 40% of controls and 41% of PD patients had hyperhomocysteinemia. As evident, there was no significant difference in the prevalence between the two groups. We also did not find any significant difference in the median homocysteine levels between patients (16.7 mg/dl) and controls (16 mg/dl) even though very few patients were on COMT inhibitors. This may be due to the normal B12 and folate levels noted in our study population. This hypothesis is supported by other studies. Kocer *et al.* compared serum homocysteine levels in PD patients on levodopa, levodopa with entacapone, and those on dopamine agonists alone and did not find any difference in the serum homocysteine levels, suggesting that levodopa may only cause a slight increase in homocysteine levels.<sup>[32]</sup>

Increasing prevalence of PN among PD patients on either oral or transjejunally infusional levodopa has been noted in the last decade and suggests a role of iatrogenic Vitamin B12 deficiency and hyperhomocysteinemia.<sup>[2,3,33,34]</sup>

Hyperhomocysteinemia may cause PN by: (1) depletion of nitric oxide increasing vasomotor tone and induction

of oxidative stress with subsequent vascular endothelial damage of vasa nervorum, (2) microthrombus formation as procoagulant – anticoagulant pathway is shifted towards coagulation, (3) direct damage to cell wall components and nucleic acid of nerve cells leading to cell necrosis or apoptosis, and (4) significant reduction of neurotrophic factor secretion by damaging Schwann cells of peripheral nerve, thus affecting cell survival.<sup>[35-37]</sup> Methylmalonic acid elevation can impair myelination of peripheral nerves.<sup>[38]</sup> Hyperhomocysteinemia may worsen PD by increasing nigral degeneration leading to increased levodopa requirement and initiation of a vicious cycle.<sup>[39]</sup> Some have advocated vitamin supplementation in PD patients to prevent these complications.<sup>[2,6]</sup>

There is a large variance in the prevalence of neuropathy in PD patients noted in different studies. Of 55 PD patients studied by Toth *et al.*, PN was present in 32 (58%), compared to 9% of controls.<sup>[2]</sup> Similarly, a high prevalence was detected by Rajabally and Martey – 14 of 37 (37.8%) patients with PD compared to 3 of 37 (8.1%) controls had PN ( $P = 0.005$ ).<sup>[3]</sup> However, in a larger cohort of 500 PD patients screened for symptomatic PN by Toth *et al.*, it was detected in 34 patients, i.e., 7%.<sup>[6]</sup> A similar prevalence was noted in our study. In 93 patients, 7 had PN (7.53%) as compared to 3 controls with PN (4.29%). The incidence of PN was not statistically different among our patients and controls. This may be explained by the variation in the methodology used. We included only symptomatic patients with abnormalities in routine NCSs. The use of more sensitive techniques such as quantitative sensory testing would have probably increased the prevalence in our study.<sup>[40]</sup> Moreover, a higher B12 level and lower homocysteine level in our population may have an additional protective effect against neuropathy.

In accordance with a recently published paper, our PD patients with PN were older (mean = 62.86 years as compared to 57.4 in PD without PN) and had longer exposure to levodopa (median duration of disease of 8 years as compared to 6 in PD without PN).<sup>[5]</sup> Among the seven PD patients with PN, 2 (28.6%) patients with PN had Vitamin B12 deficiency in contrast to 7 out of 86 PD patients who did not have neuropathy (8%). This is similar to previous studies, wherein PD patients with PN had lower B12 levels in comparison to PD patients without neuropathy.<sup>[3,5]</sup>

We could not compare the median values of serum Vitamin B12, folate, and homocysteine levels across patients with or without PN and controls as our sample was too small to compute any summary statistic.

### Limitations

Our study has few limitations. Most of the patients included in the study were from middle-income group, were well cared for by their families, and were on regular follow-up. We excluded patients who were pure vegetarians and those who were more likely to be prone to B12 deficiency and hence cannot comment on the effect of levodopa on worsening mild deficiencies. Hence, it is difficult to generalize the results to the entire Indian population.

We did not assess MTHFR genotype of the patients, their Vitamin B6 levels or fasting methylmalonic acid levels, which can influence the outcome of the study. We only included patients with symptomatic large-fiber neuropathy. We could not perform other tests for small-fiber neuropathy. The magnitude of potential confounding factors and effect modification could not be assessed by appropriate regression methods due to inadequate sample size for the purpose.

### CONCLUSION

Our study, the first from Indian PD patients, aimed to assess the prevalence of Vitamin B12 deficiency, hyperhomocysteinemia, and their association with PN and AD. In this study, we did not find a significant difference between PD patients or controls in any of the parameters. The prevalence of Vitamin B12 deficiency, hyperhomocysteinemia, and incidence of PN among our patients are very less when compared to the Western population. Hence, the conjecture that PN in PD patients may be secondary to Vitamin B12 deficiency/hyperhomocysteinemia stands as a speculation.

However, PD disease severity and longer levodopa exposure were associated with lower B12 levels and higher homocysteine levels, identifying a vulnerable group. Similarly, PD patients with PN had a higher prevalence of B12 deficiency compared to those without, emphasizing its pathogenetic role.

As B12 deficiency and hyperhomocysteinemia in PD can be induced by levodopa, albeit to a small degree, and are potentially reversible causes of PN, it is important to prevent or identify them early. Treatment modifications with addition of COMT inhibitors in our patients may also help in preventing hyperhomocysteinemia.

Especially in a setting where vegetarianism is prominent, prevention and early treatment can help PD patients avoid complications of added PN and improve their quality of life.

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### Conflicts of interest

There are no conflicts of interest.

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