

Advanced radiological investigation of levodopa-induced dyskinesia in Parkinson's disease patients: assessment of blood brain barrier function

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Abstract

Background: The purpose of this study is to explore changes in blood-brain barrier (BBB) function and volumetry associated with Parkinson's disease (PD) levodopa-induced-dyskinesia (LID). **Methods:** Twenty-six PD patients [matched pairs, 13 with LID (LID+) and 13 without (LID-)], performed high resolution 3D FSPGR MRI, applying a novel methodology developed for calculating delayed-enhancement-subtraction-maps, representing BBB function. Segmentation software calculated volumes of pre-determined brain structures and the mean BBB function was calculated for each structure. Comparisons between the LID+ and LID- paired patients and within patient, between the more and less affected hemisphere (MAH, LAH) and correlation tests with lateralized UPDRS motor scores were performed. **Results:** There were no significant differences in volumetric or BBB map characteristics between the matched LID+ and LID- patients regarding most brain areas except for the inferior parietal cortex (IPC) of the MAH that displayed a higher BBB disruption in LID+ vs. LID- patients. A positive correlation was found with the motor score of the side contralateral to the MAH ($r = 0.58$, $p < 0.038$) among the LID+ patients.

Conclusions: We demonstrated an association between slight BBB disruption in the IPC and LID in patients with PD using a new MRI methodology. As currently there is no known straightforward biological explanation for this positive finding, it might be genuine and novel, or spurious. Further studies to explore BBB functioning in the various stages of PD and its motor complications are needed, as well as further investigation of the IPC clinical importance and correction for epiphenomenon.

Keywords: levodopa-induced dyskinesia; levodopa; magnetic resonance imaging (MRI); Parkinson's disease; blood brain barrier (BBB), angiogenesis, voxel-based morphometry; parietal cortex

INTRODUCTION

Loss of dopaminergic input to the striatum of patients with Parkinson's disease (PD) results in dopamine depletion and a cascade of functional modifications that involve the basal ganglia circuitry, representing the neural substrate for bradykinesia, rigidity, and tremor. The dopamine precursor levodopa is the standard treatment for alleviation of these motor symptoms. PD patients are chronically treated with levodopa and as the disease progresses they gradually develop two clinical phenomena regarded as complications of levodopa therapy, motor fluctuations (MF) and abnormal involuntary movements termed levodopa-induced dyskinesia (LID). LID is common in advancing PD, appearing in 50% of

PD patients within 5 years of levodopa treatment.¹

The neural mechanisms underlying LID are still mostly obscure. It is assumed that abnormal neuroplastic changes are involved, originating in the striatum and leading to alterations in the firing patterns between several structures of the basal ganglia and the cortex, with disinhibition of thalamocortical neurons and over-activation of frontal areas, particularly involving the motor, premotor, and prefrontal cortices.² This process involves changes in both pre- and postsynaptic dopaminergic mechanisms with functional alterations of striatal output neurons which relates to events occurring in glutamatergic inputs from the cortex and in cholinergic and GABAergic striatal interneurons.³ Fundamental processes in

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brain plasticity involve neurons, astrocytes and microvascular cells (consisting the microvascular unit), that undergo long-lasting structural and functional adaptations.^{4,6}

Endothelial proliferation, angiogenesis and an ensuing increase in blood–brain barrier (BBB) permeability can occur in the adult human brain as an adaptation to injury and also due to locally increased metabolic demands.^{7–9}

In animal models of PD and in human PD, evidence has been demonstrated for angiogenesis and alterations in BBB function which may also be associated with inflammation.^{10–17}

Recent studies have demonstrated angiogenesis, changes in BBB function and up-regulation of vascular endothelial growth factor (VEGF) in animal LID models as well as in postmortem brain specimens of PD patients that exhibited LID^{14,16,18,19}, suggesting that neural plasticity changes leading to LID are brought out by vascular remodeling and BBB dysfunction. However imaging studies have failed to demonstrate changes in BBB function in animal models of PD with LID.²⁰

A proposed mechanism for BBB disruption in LID patients could be that microvascular or osmotic effects of L-dopa may act to transiently or chronically disrupt the BBB. L-dopa induced microvascular proliferation of immature cerebral vessels lacking a robust BBB has been proposed in rodent models.¹⁴

The aim of our study was to find evidence for BBB disruption in the brains of PD patients that developed LID, using a new MRI methodology that has been developed at Sheba Medical Center. This methodology is based on delayed contrast extravasation developed for calculating three-dimensional (3D) delayed-enhancement-subtraction-maps (BBB maps), depicting BBB function with high resolution and high sensitivity to changes. This methodology has demonstrated sensitivity to subtle BBB disruption in ischemic stroke patients and was shown to be advantageous over traditional MRI methodologies in patients with various brain tumors for differentiating malignant from non-malignant abnormal tissues (such as radiation necrosis) in neuro-oncological patients.^{21–24} In contrast to previous studies, here we studied the entire brain and not pathological lesions.^{13,15,25}

METHODS

Patient population recruitment and assessment

We designed an observational matched case-

control study of idiopathic PD patients treated with levodopa for at least 3 years, that had developed LID (LID+) and those that had not developed them (LID-), matched one-to-one for age, gender, PD duration and levodopa treatment duration; namely each LID+ patient had a matched LID-control patient “pair”. The study was approved by the local institutional review board of the Sheba Medical Center and written informed consent was obtained from all patients prior to inclusion.

Patients were identified from the PD database at the Movement Disorders Institute at Sheba Medical Center. Inclusion criteria included (1) age 30–80 years, (2) clinical diagnosis of PD according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria²⁶, (3) treatment with levodopa for at least 3 years, (4) stable medication dose for at least 4 weeks prior to recruitment and (5) reliable documentation of symptom progression from disease onset including the presence or absence of LID. Exclusion criteria included: (1) Any other brain disorder or previous surgery (inclusion deep brain stimulation or cerebral lesioning procedures for PD) (2) evidence of significant brain lesions per previous CT or MRI, (3) use of antipsychotic drugs, (4) contraindications for MRI, (5) excessive tremor or LID that would prohibit high quality MRI acquisition and (6) dementia. Patients who had developed LID at least one year prior to inclusion were defined as LID+ and those with no evidence of LID throughout their follow up visits were defined as LID-.

All patients attended a single visit in which they underwent an interview, neurological examination and MRI scan. Subjects were assessed in the “on-medication state”, namely after taking their regular antiparkinsonian medications, and severity of motor symptoms was rated using the motor section (part III) of the Unified Parkinson’s Disease Rating Scale (providing a motor score, m-UPDRS).²⁷ The lateralized m-UPDRS scores were calculated by summing together all motor items from the upper and lower limbs for each side (tremor at rest, rigidity, dexterity, consisting of items 20, 21, 22, 23, 24, 25 and 26). The more affected hemisphere (MAH) was decided upon according to documented history of side of motor symptom onset and confirmed by present neurological examination and m-UPDRS, supportive of persistent motor asymmetry. Staging of PD was determined according to the Hoehn and Yahr scale.²⁸ Severity of dyskinesia was rated using the modified abnormal involuntary movements scale (AIMs).²⁹ The levodopa daily dose (LDD,

mg) and the levodopa equivalent daily dose (LEDD, mg) were calculated for each patient.³⁰ All subjects were then scanned by standard and delayed contrast MRI, in the on-medication state.

MRI acquisition

Each MRI exam was performed in 2 parts: the first consisted of a standard brain protocol including T1-weighted fluid-attenuated inversion-recovery (FLAIR) MRI for delineating gray/white matter, T2-weighted MRI, T2-weighted FLAIR MRI, diffusion tensor MRI (DTI), gradient-echo (GE) MRI and dynamic susceptibility contrast perfusion-weighted MRI (DSC PWI) for depiction of brain anatomy and exclusion of brain abnormalities. A standard single dose (0.2 ml/Kg) of Gadolinium-DOTA (Gd, Dotarem, 0.5 mmol/mL, Guerbet, 95943 Roissy CdG Cedex, France) was injected intravenously using an automatic injection system during the DSC PWI sequence, followed by acquisition of a T1-weighted 3D fast-spoiled gradient-echo (FSPGR) sequence. Patients were then taken out of the scanner and asked to return for a short 5-10 min scan, ~70 min after contrast injection (average time = 67.6 ± 7.22 minutes). The late MRI scan consisted of the same T1-weighted 3D FSPGR sequence. MRI data acquisitions were performed using either a 1.5 T MRI system Optima MR450w (with the standard 24-channel phased array coil) or a 3.0 T MRI system Signa HDxt (with the standard 8-channel phased array coil) of GE Medical System.

MRI data analysis

The overall goal of the analysis was to obtain BBB maps, where the 3D FSPGR-MRIs of the first series post contrast were subtracted from that of later series. These maps depict spatial distribution of contrast accumulation/clearance in the tissue (where the signal is averaged in each 1 mm³ voxel over the tissue and microvasculature), blood vessels and CSF. The signal decay of the blood vessels is faster than that of the tissue, therefore blood vessels have lower values than tissue. In case of intact BBB, due to clearance of contrast agent from the blood system, the signal decays with time; therefore the subtraction maps have negative values. In case of BBB disruption leading to leakiness, there is accumulation of contrast interstitially, causing a signal increase, thus the values in these regions are positive. In order to enable comparison between maps of different patients, the SI in the maps had to be normalized.

The strongest signal decrease between the 2 MRI series is measured in the blood vessels (which consist of the highest contrast concentration post injection), and therefore the BBB maps were normalized by defining 30% signal loss in the blood vessels to be -1. As a result, all voxels showing clearance of contrast in the maps have values between 0 and -1. Voxels showing contrast accumulation have values between 0 and +1. In order to increase the sensitivity to small changes it was essential to perform image pre-processing prior to subtracting the two MRI series, consisting of corrections for intensity variations and whole body image registration, as previously described.^{21,23,24}

The whole post-processing is performed under the MATLAB environment (The MathWorks, Inc. Natick, MA, US). The steps needed to reconstruct the 3D TRAM Include high precision 3D whole brain registration, based on the least square approach and a 6 parameter (rigid body) spatial transformation available in the SPM toolbox. Then CSF-weighted whole brain registration is performed using decreasing kernel width “Full Width Half Max” (FWHM) and 1 cm sliding slab CSF-weighted local registration. Radio-frequency inhomogeneity correction is performed with 3D Gaussian filtering on both registered images and automatic dynamic normalization is done with signal span assessed for each patient based on automatic detection of the strongest Gadolinium decrease within the image i.e. vessel voxels. Normalized 3D TRAM are computed accordingly.

In order to determine volumes of interest (VOIs) of the various brain structures, FreeSurfer 5.3 was used, (<http://surfer.nmr.mgh.harvard.edu>), allowing a semi-automated anatomic segmentation and voxel-based morphometry (VBM) for the volumetric analyses. Segmentation of cortical structures, subcortical white matter and deep gray matter structures, and volumetric analysis were performed. Images obtained for each structure were inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. *Bbregister*, an intrinsic FreeSurfer tool, was used, to provide accurate and robust brain image alignment using boundary-based registration³¹ in order to match the 3D BBB maps to the native space of each structure in the FreeSurfer environment. The registration process allowed utilizing the FreeSurfer anatomical brain segmentations for further calculations of mean intensities for each VOI from the 3D BBB maps.

Once VOIs were determined for the different

brain segments using FreeSurfer, the volumes of the VOIs were calculated by adding the number of voxel in each VOI and multiplying by the volume of a single voxel. In addition, the average SI and standard deviation were calculated for each VOI from the BBB maps.

Statistical methods

The mean BBB SI and the volumetric data of all FreeSurfer anatomical VOIs, predetermined brain segments, were compared between matched LID+ and LID- PD patient pairs [comparing the MAHs in both groups or the less affected hemispheres, (LAHs)] using the non-parametric Wilcoxon-Mann-Whitney test.³² Additionally the average of absolute value differences between LID+ and LID- patients was calculated, to determine “normal” variability in these measures.

The mean SI and the volumetric data of each VOI in the MAH, were compared within patients to those of the LAH separately for the LID- and for the LID+ groups as well as for the whole cohort, using the non-parametric sign test.³²

Spearman correlation analysis was used to study correlations between the mean BBB SI and the volumetric measurements of the anatomical VOIs in the MAHs and in the LAHs, and ten clinical features (gender, age, age of PD onset, levodopa treatment duration, LDD, LEDD, m-UPDRS score, respective lateralized m-UPDRS score, AIMS score and Hoehn and Yahr stage) both for the LID+ and LID- groups, and in the whole cohort.³²

All statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). The level of significance for the comparison between the two groups was $p < 0.05$. Due to the explorative nature of the study a correction for multiple analyses was not done.

RESULTS

Thirty-five PD patients (19 LID+) were recruited, examined and scanned according to the protocol. Segmentation was performed and BBB maps were calculated for all VOIs.

Nine patients were excluded due to violation of inclusion criteria (change in diagnosis to multiple system atrophy, $n=1$) or due to incomplete MRI protocol [due to technical problems ($n=4$), poor quality images ($n=1$), or interrupted scan because of claustrophobia ($n=3$)].

Patient characteristics

Data obtained from 26 PD patients treated with

levodopa (10 females, age 58.9 ± 5.4 years, disease duration 8.5 ± 3.5 years, levodopa treatment duration 5.7 ± 2.7 years), representing 13 matched pairs of LID+ and LID- patients, were analyzed. There were no significant differences between the groups in demographical (age, gender) and clinical data (age at PD onset, PD duration, levodopa treatment duration, m-UPDRS, and lateralized m-UPDRS score of the more affected side); However the LDD and LEDD were significantly higher for the LID+ group than for the LID-group. (Table 1)

MRI derived data

The results of the average BBB SI in some of the several structures studied, pertaining to the MAH, are presented in Table 2. A statistically significant difference was found between the matched LID+ and LID- pairs when comparing SI of the MAH; for the LID+ patients, the inferior parietal cortex (IPC) displayed a less negative BBB SI than the LID- group, 6% difference ($p < 0.01$), while the average of absolute value differences between LID+ and LID- patients was $3\% \pm 2.1\%$ (SD=noise level) (Table 2). In 10 out of 13 couples this was evident on an individual basis (Figure 1). This statistically significant difference shows that the LID+ group has reduced contrast clearance, suggesting higher BBB permeability in the IPC of the MAH, relatively to the LID- group (Figure 2).

There was no difference in the volume of the IPC of the MAH between LID+ and LID-.

No correlations were found between BBB SIs and gender, patient age, or AAO, levodopa treatment duration, LDD, LEDD, m-UPDRS score, lateralized m-UPDRS scores, AIMS score and Hoehn and Yahr stage, for the whole group or for the LID- group in any of the structures studied neither in the MAHs and nor in the LAHs. For the LID+ group a positive correlation was found between BBB SI of the IPC of the MAH and the m-UPDRS score ($r = 0.58$, $p < 0.038$) and also lateralized m-UPDRS score ($r = 0.55$, $p = 0.05$). This was not found for the LAH (data available in supplemental materials).

There were no significant differences in volumetric measurements of most brain segments analyzed by VBM, between the LID+ and LID- matched patient pairs, in neither the more, nor the LAHs. The volumes of some of the brain segments of the MAHs of the LID+ and LID- matched patients are presented in Table 3. A statistically significant difference was found pertaining to the superior parietal cortex (SPC) of the MAH which was larger in the LID- group than in the LID+

Table 1: Demographic and clinical characteristics of PD patients, LID+ and LID- matched pairs

	LID+ (n=13)	LID- (n=13)	P value*
Gender (female/male)	5/8	5/8	1
Age (years)	59.2 ± 5.8	58.7 ± 5.3	0.8
Age at PD onset (years)	50.5 ± 5.8	50.5 ± 7.2	0.9763
PD duration (years)	8.6 ± 3.2	8.3 ± 3.9	0.8268
L-dopa treatment duration (years)	6.2 ± 3.1	5.2 ± 2.2	0.3125
L-dopa daily dose (mg)	683 ± 349	416 ± 253	0.0356
L-dopa equivalent daily dose (mg)	1036 ± 366	711 ± 272	0.0168
M-UPDRS score	31.0 ± 11.2	40.4 ± 17.1	0.1112
Lateralized m-UPDRS score (more affected side)	8.7 ± 5.4	10.2 ± 5.4	0.579
AIMS score	14.4 ± 8.5	-	-
Hoehn and Yahr Stage**	2.5 (2.0-2.5)	2.75 (2.0-3.0)	0.2461

LID+= patients that developed levodopa-induced dyskinesia

LID-= patients that had not developed levodopa-induced dyskinesia

*Comparisons are performed between LID+ and LID- groups

All data present mean± standard deviations **Median (interquartile range).

M-UPDRS= motor section (part III) of Unified Parkinson's Disease Rating Scale

AIMS= modified abnormal involuntary movements scale

group (12% difference, $p < 0.039$). The average of absolute value differences was $5.1\% \pm 5\%$.

There were no significant correlations between the SPC volume and any of the clinical features studied.

No differences were found between groups

regarding SIs and volumetric measurements of other or midline structures.

When comparing BBB SIs of the MAH to the LAH within-patients, no difference was found including for the IPC or SPC.

In the frontal pole (part of the prefrontal cortex),

Table 2: Comparison of mean BBB signal intensity ratio of anatomical brain structures between LID+ and LID- matched pairs (concerning the more affected hemisphere)

Brain structure	LID + (N=13) IQR	LID - (N=13) IQR	P value
Superior parietal cortex	-9.1% (-14.7%--4.5%)	-15.0% (-16.9%--8.3%)	0.1465 ^a
Inferior parietal cortex	-10.6% (-12.1%--6.7%)	-16.6% (-19.5%--10.4%)	0.0134 ^a *
Cerebellar cortex	-24% (-27.7%—7%)	-19% (-36.4%--12.3%)	0.5417 ^a
Thalamus proper	-11.4% (-14.4%--10%)	-12.8% (-15.8%--11.4%)	0.5879 ^a
Caudate	-19.2% (-20.8%--15%)	-14.6% (-16.6%--11%)	0.3054 ^a
Putamen	-11.9% (-15.4%--9%)	-11.3% (-13.1%--9.1%)	0.7354 ^a
Pallidum	-8.1% (-11%--6%)	-10.3% (-11.1%--5.6%)	0.4548 ^a
Superior frontal cortex	-13% (-15%--9.1%)	-7.1% (-12.8%--3.5%)	0.3396 ^a
Frontal pole cortex	-10.5% (-14.0%--3.6%)	-6.0% (-14.1%--0.0%)	0.6848 ^a
Precentral cortex	-14.8% (-19.5%--10.6%)	-13.5% (-17.0%--12.2%)	0.7354 ^a

Data are given as median values (interquartile range- IQR); The mean BBB signal intensity ratio are the measures of gadolinium clearance ratio (-) or accumulation ratio (+) obtained by the delayed enhancement subtraction maps for each segment obtained by the FreeSurfer platform, reflecting BBB function. The average of absolute value differences of Gd intensity ratios between LID+ and LID- pertaining to all brain segments for all patients was $3\% \pm 2.1\%$

^aMann-Whitney test

*Statistically significant

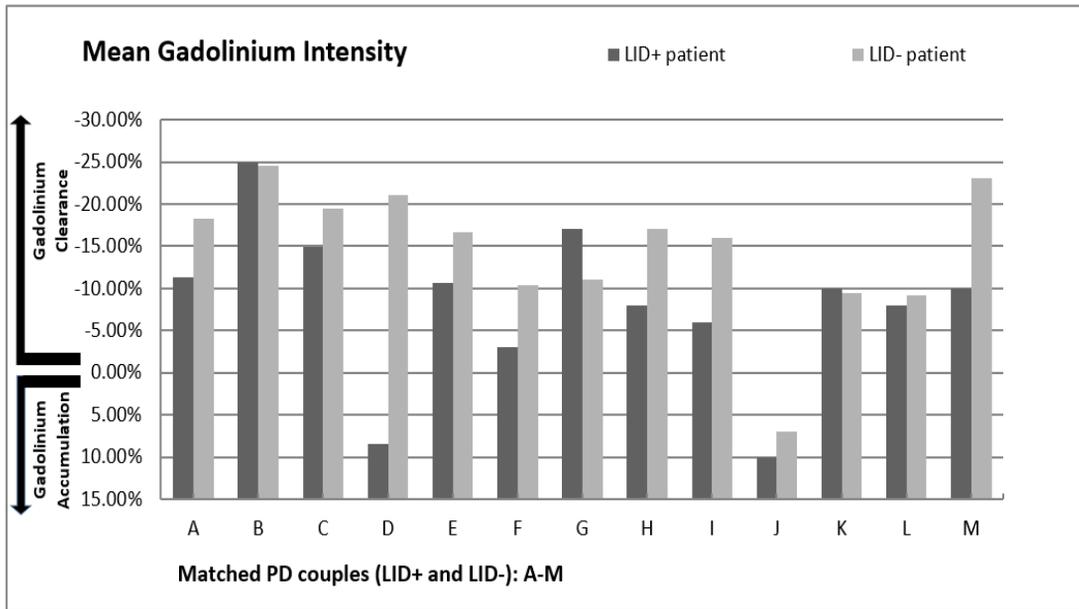


Figure 1. Average Gadolinium intensity ratio in the inferior parietal cortex of PD patients, LID+ and LID- matched pairs (in the more affected hemisphere)

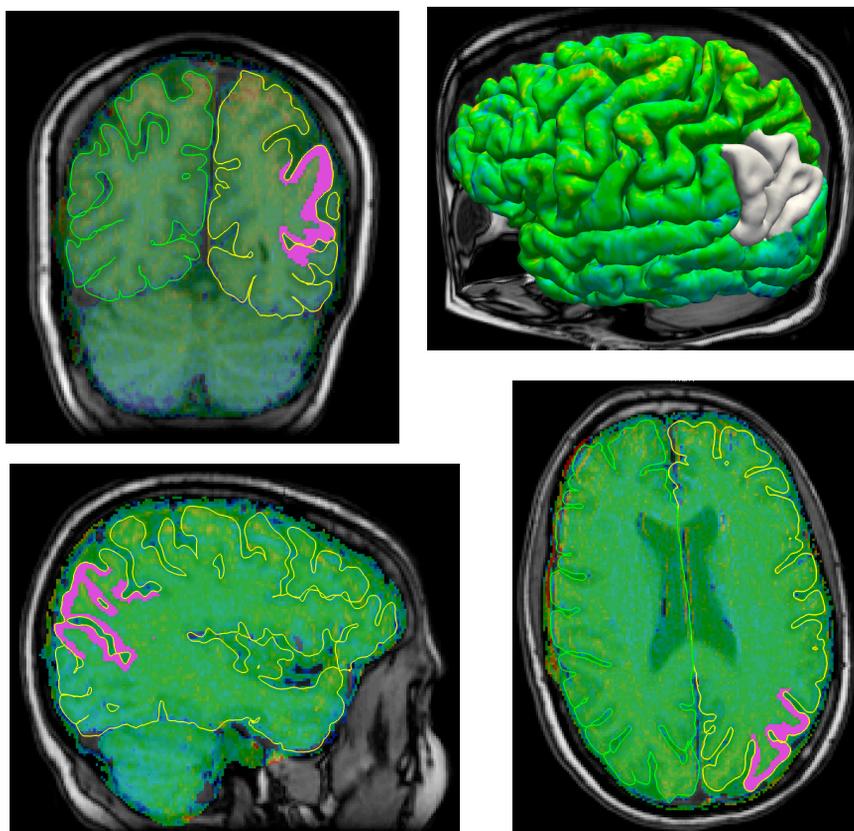


Figure 2. Three dimensional (3D) illustrations suggesting higher BBB permeability in the inferior parietal cortex (IPC). This figure demonstrates a reduced contrast clearance in the IPC of the more affected hemisphere in the LID+ group relatively to the LID- group ($p < 0.01$), suggesting higher BBB permeability in the IPC in patients with dyskinesia.

Table 3: Comparison of volumes of anatomical brain structures between LID+ and LID- matched pairs (concerning the more affected hemisphere)

Brain structure	LID+ (N=13) IQR	LID- (N=13) IQR	p-Value
Superior parietal cortex	10585.0 (10119.0-13063.0)	11994.0 (10496.0-12470.0)	0.0398 ^{a*}
Inferior parietal cortex	11882.0 (9358.0-15153.0)	12263.0 (11124.0-15475.0)	0.1272 ^a
Cerebellum Cortex	51993.0 (48522.0-53833.5)	44814.0 (42744.0-51279.5)	0.2734 ^a
Thalamus proper	7704.0 (7435.6-8463.0)	8451.0 (7284.0-8969.0)	0.7354 ^a
Caudate	3143.0 (2302.0-3305.0)	3070.9 (2766.0-3338.0)	0.8926 ^a
Putamen	4389.8 (3681.0-4841.0)	4548.0 (4234.0-4697.0)	0.4973 ^a
Pallidum	1435.0 (1017.0-1619.0)	1415.0 (1227.0-1633.0)	0.5879 ^a
Superior frontal cortex	18832.0 (17965.0-19903.0)	18754.0 (16617.0-21157.0)	0.7354 ^a
Frontal pole cortex	932.0 (753.0-1097.0)	1179.0 (921.0-1192.0)	0.5417 ^a
Precentral cortex	11878.0 (10995.0-12291.0)	11957.0 (10548.0-12904.0)	0.6355 ^a

Data are given as median values (interquartile range- IQR): The measures are volumes=number of voxels (mm³) - obtained for each anatomical segment by the FreeSurfer platform.

^aMann-Whitney test

*Statistically significant

the BBB SI was higher (less negative) in the more affected than the LAH (6% difference, $p < 0.01$) in the whole cohort and in the LID+ group (6% difference, $p < 0.04$), but not for the LID- group. The average of absolute value differences between the MAHs and LAHs was $1.7\% \pm 1.4\%$ (data available in supplemental materials).

No difference was found in volumetric measurements comparing MAH to the LAH within-patients, both in the whole cohort (N=26), and within the LID+ and LID- patient groups (N=13 in each).

DISCUSSION

While it is clear that two requirements are necessary for the induction and maintenance of LID: severe dopamine denervation in the striatum and pulsatile exposure to high levodopa doses, the mechanisms involved in the pathophysiology of LID have not yet been fully elucidated.

We performed a neuroimaging study with focus on BBB dysfunction and volumetric changes in 13 PD patient couples that were all treated with levodopa, matched for PD duration and other factors but differed in LID status. Our study is the first MRI study in humans, assessing BBB permeability in PD LID.

Using a novel MRI methodology combined with automated segmentation software we could detect only minor subtle changes in BBB function and volumetric measures in a few brain structures that did not stand correction for multiple analysis. We found evidence for higher BBB permeability

in the IPC of the MAH in LID+ patients, relative to the LID- patients. Furthermore, for the LID+ patients, this score correlated positively with the severity of their motor symptoms. This could suggest that in PD, BBB disruption may lead to LID as parkinsonian motor symptomatology worsens. The absence of an accompanying change in IPC volume and the absence of differences in BBB function between the more and less affected hemispheres do not support the association suggested between IPC BBB disruption and LID.

The finding of a slightly but significantly smaller SPC volume in patients with LID than those without remains isolated and poorly explained.

There have been a number of investigations on LID in PD that pointed towards a role of the basal ganglia and frontal motor areas (e.g., prefrontal cortex) in the pathogenesis of LID. However, the IPC has only been remotely related to LID. Animal studies have provided strong evidence for a central role of the putamen and its cortical projections in LID.^{33,34} A study of unilaterally lesioned 6-hydroxydopamine rats treated with levodopa that developed dyskinesia, a regional flow-metabolism dissociation and increased BBB permeability were induced within the Striatum/Globus Pallidus (GP) in areas of active microvascular remodeling, and that such changes correlate with the severity of dyskinesia.¹⁸ MRI studies have additionally identified cortical regions playing key roles in the development of LID comprising the supplementary motor area

(SMA)³⁵⁻³⁷, primary sensorimotor cortex (SM1)^{37,38} and right inferior frontal cortex (IFC).^{25,35,39} There are some inconsistencies, though, between these studies regarding the anatomical structures involved in the development of LID. Within several studies Cerasa and colleagues have established the role of the prefrontal cortex as a key site of importance, demonstrating that, among other areas, the IFC is particularly characterized by altered patterns of anatomical and functional changes.³⁸ When compared with LID- patients, LID+ patients showed increased IFC volume and a dysfunctional imbalance between this region and the SMA during motor task. We did not find an effect of LID status on the volume of the IFC or other structures (apart from the slight difference in the superior parietal cortex) analyzing the matched pairs. Leaving that aside, it has been suggested in the literature that the pathogenesis of LID also involves limbic, cognitive and associative structures including parietal areas.^{33,40}

It is hard to explain involvement of parietal regions in LID, first being that there are hardly any dopaminergic receptors in this area (as opposed to the basal ganglia or frontal cortex), and the development of LID in PD appears to be inheritably dependent on dopaminergic metabolism. If validated in further studies perhaps a new pathogenetic path should be considered, unrelated to dopaminergic metabolism. Another possibility is its being an epiphenomenon, not related to LID but rather to more advanced neurodegeneration in LID+ vs LID- patients.

As these findings would not be detectable if we had corrected for multiple analyses, we could state that our methodology combined with brain segregation for assessment of BBB function in discrete brain structures, did not find firm support for the idea that neural plasticity changes leading to LID are brought out by vascular remodeling and BBB dysfunction. Not much support has been gathered for the angiogenetic LID hypothesis up to now. An animal model imaging study did not support the theory of BBB disruption in LID, assessing BBB integrity in vivo MRI in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned macaque monkeys exhibiting LID; They performed MRI before and immediately after injection of a Gd-based contrast agent and revealed an intact BBB in the basal ganglia²⁰ and concluding that LID was not associated with a disrupted BBB in that model. However, the method employed might have failed to detect BBB dysfunction due to the low-sensitivity to subtle BBB disruption.

There are a number of limitations to our study. First, this novel MRI methodology has never been explored in PD or other neurodegenerative disorders (such as Alzheimer's disease, amyotrophic lateral sclerosis, or chronic traumatic encephalopathy) in comparison to healthy control subjects. It is to be shown if this novel imaging methodology for whole brain BBB function can detect subtle permeability alterations in vivo in patients providing support to the neuropathological changes that had been previously demonstrated in these disorders.^{41,42} Second, we included a small sample size, as it was an exploratory pilot study and we had no healthy control group. Furthermore the L-dopa daily dose was a point of statistical difference between the two (LID+ and LID-) could potentially explain the differences in BBB function found. Furthermore, the absence of correction for multiple comparisons in the frame of the exploratory design is again noted, given the high number of comparisons and correlations performed.

Another limitation is the lack of multivariate analysis of confounding variables, as duration and severity of LID could potentially affect the comparisons between LID+ vs LID- patients.

Future studies are needed on BBB dysfunction in PD as well as in other neurodegenerative disorders and conditions where BBB disruption is suspected in comparison to healthy controls. It is important to explore BBB function along the various phases of PD in the context of disease progression, with a focus on motor and other complications, applying specific VOI with larger patient samples with and without LID, possibly in a matched-case design similar to the present study.

In conclusion, in this pilot study, we used an innovative advanced MRI methodology to explore changes in BBB function and their correlation with volumetric measurements, in association with LID in PD patients. While the basal ganglia and frontal areas did not manifest BBB or volumetric changes in association with LID, a weak association of slightly higher BBB disruption was found in the IPC in patients with LID. As currently there is no known straightforward biological explanation for this positive finding, it might be genuine and novel, or spurious. Further studies to explore BBB functioning in the various stages of PD and its motor complications are needed, as well as further investigation of the IPC clinical importance and correction for epiphenomenon.

DISCLOSURE

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of our institutional research committee in Sheba Medical Center and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients that have participated in this study have signed written informed consent forms in accordance with Helsinki declarations. The institutional research committee's reference number in Sheba Medical Center for this study is 971912SMC.

Availability of data and materials: All data can be found in the movement disorders institute in Sheba Medical Center, Israel. For individual privacy, no link can be provided. If further information is needed, please contact ophirsg@gmail.com. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest: None

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