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Publication details:
Movement Disorders
v. 27
Chapter No. 12
pp. 1506-1512
0885-3185 (ISSN)

Publication Date:
2012

Publisher DOI:
http://dx.doi.org/10.1002/mds.25112

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Small-vessel disease in patients with Parkinson Disease: a clinicopathological study

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Keywords: stroke, neuropathology, vascular risk factors

Word count: 3000

Financial Disclosure/Conflict of Interest related to the research covered in the article: GH is funded as a Senior Principal Research Fellow of the National Health and Medical Research Council of Australia to perform research. The authors declare that they have no conflicts of interest.
Abstract:

Few studies have examined the relationship between cerebrovascular disease, vascular risk factors and Parkinson disease (PD), although one study found small vessel disease (SVD) to be the main subtype of cerebrovascular disease. In this study we compared the extent and topography of SVD and assessed associated vascular risk factors in autopsy-proven PD cases and community-dwelling controls. 77 PD and 32 control brains from the Sydney Brain Bank were assessed microscopically by a single examiner blinded to the diagnosis. SVD was assessed by grading perivascular pallor, gliosis, hyaline thickening and enlargement of perivascular spaces in the white matter underlying the superior frontal and primary motor cortices, basal ganglia and white matter tracts. A history of vascular risk factors (hypertension, heart disease, diabetes and cigarette smoking) was obtained. Groups were compared using stepwise multiple regression analysis. There was significantly greater frontal pallor (p=0.004) and widening of perivascular spaces in the globus pallidus interna (p=0.007) in controls compared with PD. Hyaline thickening and widening of perivascular spaces in the frontal white matter, hyaline thickening in the motor white matter and widening of perivascular spaces in the caudate nucleus were more common in the control group, but did not reach significance. The prevalence of vascular risk factors and SVD pathology was significantly lower in autopsy-proven PD compared with controls (p=0.03) living in the same community. The results of this study support the need for further research in this area.
Background

Few studies have examined the relationship between cerebrovascular disease (CVD), vascular risk factors and Parkinson disease (PD) despite evidence that the prevalence of CVD in PD may be ten times more common than vascular parkinsonism {Hughes, 1992 #1334}. Clinical, radiological and pathological studies examining CVD in PD have revealed variable results, with some studies demonstrating a small increase in prevalence {Gorell, 1994 #612; Ben-Shlomo, 1995 #611; Becker, 2010 #1333; Piccini, 1995 #1369; Gattellaro, 2009 #610}, some a small decrease {Jellinger, 1990 #1349; Nataraj, 2005 #1361} and others a similar prevalence {Mastaglia, 2002 #1351; Jellinger, 2003 #1252; Ghebremedhin E, 2010 #1054; Levine, 1992 #1353; Beyer, 2006 #1376}. A recent review of the relationship between PD and CVD {Nanhoe-Mahabier W, 2009 #1332} suggests that overt CVD is relatively rare in PD, with the type of study influencing the outcome (increased prevalence in radiological and postmortem studies compared with epidemiological studies). The authors suggest that patients with PD possibly have an increased risk of developing comorbid CVD compared with the general population and that this could alter the clinical presentation and treatment response of PD {Nanhoe-Mahabier W, 2009 #1332}. Some of the factors thought to contribute to this increased risk include decreased mobility, increased incidence of smoking and hypotension {Nanhoe-Mahabier W, 2009 #1332}.

Three studies of pathologically-confirmed PD have examined the prevalence of CVD pathology {Aarsland, 2004 #1175; Jellinger, 2003 #1252; Ghebremedhin, 2010 #1054}. These studies, which are important because of the certainty of diagnosis, all demonstrated a similar prevalence of CVD in PD and controls. A clinicopathological study of the accuracy of clinical diagnosis in PD found that striatal lacunar infarction was three times more common than cortical infarction in pathologically-confirmed PD {Hughes, 1993 #1224}. This suggests that small vessel disease (SVD) rather than cortical infarction or haemorrhage is the predominant stroke sub-type in PD. The aim of our study was to evaluate the extent and topography of SVD and associated vascular risk factors in autopsy proven PD brains versus normal controls.

Methods

Case selection and characterisation

This study examined all 77 autopsy-confirmed and eligible PD cases from a total of 141 PD brains donated to the Sydney Brain Bank (SBB) from 1996 to 2006. Neuropathological changes have been previously studied in many of these cases {Halliday, 2008 #1188}. PD cases were not excluded if they had co-existing CVD or limited Alzheimer-type pathology, however cases were excluded from our study if they fulfilled NIA-Reagan criteria {, 1997 #1056} for Alzheimer’s disease, demonstrated evidence of other neurodegenerative disorders or had insufficient clinical data to allow analysis.
Similarly, 32 community-dwelling and eligible controls from a total of 78 donors collected over the same period were selected for study. Controls had no history of dementia or movement disorder. Similar to PD cases, controls with CVD or limited, age-related Alzheimer-type pathology were not excluded and only those with other pathologies or who fell well outside the age range of PD cases were excluded.

Cases collected by Sydney Brain Bank (SBB) are from donors for whom prospective consent for use of tissue for research is gained from the participant and/or their next-of-kin. SBB holds ethics committee approval from the Southern Sydney and Illawarra Area Health Service and the University of NSW. This study was approved by the human research ethics committee of the University of Sydney. Brains were removed within 72 hours of death and either fixed by immersion in 15% buffered formalin, or hemisected and then one hemisphere fixed. After 14 days fixation, hemispheres were embedded in agar and sectioned in the coronal plane at 3mm intervals. Each slice was photographed and standard blocks taken for routine neuropathological examination. Samples from the prefrontal, primary motor, anterior cingulate and inferior temporal cortices, hippocampus, basal ganglia, midbrain, pons, medulla oblongata and cerebellum were embedded in paraffin, sectioned at 10μm intervals and stained with haematoxylin and eosin. Selected sections were impregnated with silver or stained immunohistochemically for tau, ubiquitin and alpha-synuclein. Neuropathological classification was performed according to consensus criteria {Halliday, 2002 #1071}. For this study the diagnosis of PD was made according to consensus criteria {Dickson, 2009 #2131}. In addition to diagnostic category, the Braak Parkinson disease stage {Braak, 2003 #1081}, Braak neurofibrillary tangle (NFT) stage {Braak, 1995 #1060} and the semi-quantitative CERAD neuritic plaque density score were recorded for each subject {Mirra, 1997 #2124}. The size, location and estimated age (acute, subacute, remote) of infarcts were determined from photographs of the cerebral hemisphere(s). The presence, age and anatomical location of microscopic infarcts was recorded following examination of the haematoxylin and eosin stained sections of the ten routinely screened regions listed above.

Clinical details
Clinical information was derived from the SBB database comprising results from longitudinal questionnaires obtained from referring neurologists and geriatricians including age, gender, cause of death from death certificate, medication (anti-hypertensives, statins, dopamine therapy), vascular risk factors (hypertension, heart disease, diabetes mellitus), alcohol history (number of standard drinks/day), smoking history (no=never; yes=past or current), dementia history (yes/no), falls history (never, early, late) and stroke history (yes/no). Cause of death was divided into vascular (myocardial infarction, cardiac failure, CVD and venous thrombosis/pulmonary embolism) and non-vascular.
Tissue preparation

Additional blocks were sampled from the superior frontal gyrus (Brodman area (BA) 9), precentral gyrus (BA4) and subcortical grey matter at the level of anterior amygdala, including caudate nucleus, putamen, internal capsule and globus pallidus and paraffin-embedded. Sections were cut at 10µm and stained with haematoxylin and eosin using routine methods.

Assessment of SVD

The histopathological assessment was undertaken by a single examiner (RS - neurologist) blinded to the diagnosis following training by a neuropathologist (JK). The severity of SVD was determined using a semi-quantitative scoring system (nil, mild, moderate or severe; figure 1) modified from Ziljmans et al. (Zijlmans, 2004 #688). Individual pathological features (perivascular pallor, gliosis, hyaline thickening and enlargement of perivascular spaces) were scored in each of seven regions studied viz. white matter underlying the superior frontal (frontal WM) and primary motor (motor WM) cortices, putamen (Put), globus pallidus externa (GPe), globus pallidus interna (GPi), caudate nucleus (CN) and white matter tract of the internal capsule (WMT) (table 1; figure 1). The prefrontal white matter and white matter underlying the primary motor cortex were selected as those regions are implicated in impaired motor function in vascular parkinsonism. As SVD is generally a symmetrical syndrome, only one cerebral hemisphere was examined in each case. Each SVD grading criterion was scored in five separate fields in the frontal and motor WM and in the entire section of the GPe, GPi, CN, Put and WMTs under light microscopy (x400 magnification). For the deep grey matter assessment of the severity of SVD was made in the same way as described above. However, as there are considerably more vessels in the deep grey matter, a representative proportion of the number of vessels affected by SVD in the grey matter was recorded as a percentage (in quartiles) of the overall number of vessels in the section. Ten sections were fully re-rated as outlined in the above protocol (i.e. 4 SVD criteria assessed in frontal white matter) to test intra-rater reliability. Intra-class correlation coefficient (Cronbach’s alpha) was 0.575 for gliosis, 0.918 for perivascular pallor, 0.863 for hyaline thickening and 0.555 for widening of the perivascular space (overall value 0.754).

For the frontal and motor WM regions, the average of each SVD score in each field was determined by summing the SVD score for each field and then dividing by the maximum possible score (total number of fields X 3) for each SVD parameter for each region (range of scores 0-1). For each of the five deep grey matter regions studied, the average SVD score (0-3) for each SVD parameter was multiplied by the percentage of vessels affected by SVD in each region (range of scores 0-1).
Statistical analysis

Group means for demographic variables were compared using analysis of variance with Bonferroni correction (p < 0.025) or Chi square test. A comparison of the extent and topography of SVD between PD and control brains was undertaken using multiple stepwise logistical regression analysis (SPSS v19; SPSS Inc.). Age, number of vascular risk factors, presence of stroke and Braak NFT score were entered as covariants.

Results

The mean (+SD) age at death in the PD group was slightly younger than the control group (78±7y compared with 82±13y), although this failed to reach significance (table 2). The PD group comprised over twice as many males as females compared with the control group in which the number of males and females was similar, although this difference was not significant. The mean duration of disease in the PD group was 12±7 years. Seventy of the 77 PD patients were treated with and responsive to L-dopa therapy, six had not received treatment with L-dopa and treatment data were missing for one patient. Dementia clinically affected 64% of patients, but did not pre-date the diagnosis of PD in any patient. The postmortem interval did not differ significantly between the two groups. Cause of death in both groups was predominantly non-vascular while vascular deaths composed approximate one quarter of each group. No significant difference in the frequency of the cause of death was found. Of the 77 patients in the PD group, 32 were in Braak PD stage 4; 32 in stage 5 and 13 in stage 6. The majority of cases in both groups had a Braak NFT score of 0 (84% controls and 73% PD). Limbic NFTs were seen in 16% of controls and 25% of PD cases, while neocortical NFTs were found in only 2 PD cases. Similarly, the majority of both the control (100%) and PD (82%) groups had either no or sparse plaques according to the CERAD rating (Mirra, 1991 #2127). Frequent plaques were found in four PD patients.

Evidence of infarction was observed in 16 of the 77 PD (21%) and 9 of the 33 (28%) control cases. These comprised cortical infarcts in 7 PD and 2 control cases, white matter infarcts in 2 cases in each group and basal ganglia infarcts in 10 PD and 6 controls cases. Due to the low prevalence of infarcts these were classified as present or absent for further analysis.

The total number of vascular risk factors was greater in the control group compared with the PD group (table 2; p=0.03). There was no difference in the number of vascular risk factors between males and females for either group. Of the vascular risk factors, heart disease was significantly more prevalent in control patients compared with PD patients (p<0.0001). The prevalence of hypertension and diabetes mellitus did not differ significantly between the two groups. A history of cigarette smoking was more prevalent in the PD patients but did not reach significance.
Of the pathological variables scored, mild gliosis was the most common and least discriminating finding and was therefore excluded from the multivariate analysis. Hyaline thickening was the least common finding across both groups. Most pathological features were mild or moderate, with severe changes in any rating rarely observed.

Results of step-wise multiple regression analysis covarying for age and Braak NFT score revealed a significantly increased mean number of vascular risk factors accompanied by increased SVD pathology characterised by frontal pallor and widening of the perivascular space in the GPi in the normal control group compared to PD group (table 3). Excluding PD patients without L-Dopa therapy did not alter this finding. To determine if the increased severity of SVD pathology in controls was solely as a result of the increased prevalence of vascular risk factors, a secondary analysis was undertaken in which PD and control cases were matched for age (within 2y) and number of vascular risk factors. Results from the multiple regression analysis, using the covariants listed above, showed that the increase in frontal pallor remained significant (p=0.001) while widening of the perivascular space in the GPi reduced to a trend (p=0.079). No other variable showed significance in this analysis.

Hyaline thickening and widening of the perivascular space in the frontal WM, hyaline thickening in the motor WM and widening of the perivascular space in the caudate nucleus were more common in the control brains but did not reach significance. Further analysis of the individual vascular risk factors revealed the increased prevalence in the control group was primarily due to the increased prevalence of heart disease (table 2).

Discussion
Our study examined SVD in pathologically-confirmed PD and controls from the same community. The data demonstrate a significantly greater prevalence of SVD in the community-dwelling control group associated with an increased number of vascular risk factors, and heart disease in particular. Relatively few studies have specifically examined the prevalence of CVD in PD {Mastaglia, 2002 #1351;Jellinger, 2003 #1252;Ghebremedhin E, 2010 #1054;Jellinger, #847;Levine, 1992 #1353;Beyer, 2006 #1376;Gorell, 1994 #612;Ben-Shlomo, 1995 #611;Becker, 2010 #1333;Piccini, 1995 #1369;Gattellaro, 2009 #610;Struck, 1990 #1349;Nataraj, 2005 #1361} and most of these studies have been clinical and/or radiological studies {Levine, 1992 #1353;Beyer, 2006 #1376;Gorell, 1994 #612;Ben-Shlomo, 1995 #611;Becker, 2010 #1333;Piccini, 1995 #1369;Gattellaro, 2009 #610;Struck, 1990 #1349;Nataraj, 2005 #1361}. Most have demonstrated a similar {Mastaglia, 2002 #1351;Jellinger, 2003 #1252;Ghebremedhin E, 2010 #1054;Levine, 1992 #1353;Beyer, 2006 #1376} or slightly higher {Gorell, 1994 #612;Ben-Shlomo, 1995 #611;Becker,
2010 #1333; Piccini, 1995 #1369; Gattellaro, 2009 #610) prevalence of CVD in PD. Few have shown a lower prevalence {Jellinger, #847; Struck, 1990 #1349; Nataraj, 2005 #1361}.

Our study population comprised 77 brains of patients fulfilling pathological criteria for PD with the majority being L-Dopa responsive and Braak PD stage 5 or 6 {McKeith, 2005 #1057; Braak, 2003 #1081}. We specifically excluded atypical parkinsonian syndromes, but did not exclude CVD or limited Alzheimer-type pathology. The findings of this study are in contrast to previously published pathological studies which demonstrated no difference in prevalence of CVD in patients with PD {Jellinger, 2003 #1252; Aarsland, 2004 #1175; Ghebremedhin, 2010 #1054}. However, the results of this study are consistent with a study which found a lower prevalence of CVD overall in brains from patients with Lewy body dementia compared with PD and control brains, although this difference was not significant {Jellinger, #847}. Given that our patient population comprised a majority of cases with higher Braak PD scores, the similarity with this study might be expected.

Our study is a retrospective clinicopathological study relying on clinical data collected under the auspices of the prospective brain donor program that recorded longitudinal information from participants living in the community. This differs from many hospital or forensic brain collections and studies. While our study may lack the veracity of a prospective clinical study, the clinical information is comprehensively and rigorously recorded facilitating collection of specific and accurate details of the patient demographics and vascular risk factors required for the purposes of this study, and the cases are pathologically proven with alternate diagnoses excluded. We acknowledge the potential for sample bias associated with clinicopathological studies in PD such as ours which are reliant on analysis of brain tissue from organ donors. However, compared with clinical studies, in which the precise extent of cerebrovascular and other age-related pathologies may not be known, our study of neuropathologically screened brains should have greater power to observe any true effects.

Case selection in the control group may also introduce bias into clinicopathological studies of CVD in PD {Nanhoe-Mahabier W, 2009 #1332}. In the present study, the cases and controls were collected over the same time period from prospectively studied subjects enrolled in a brain donor program. All came from a similar community population unlike other postmortem studies which are largely retrospective assessments of institutional autopsies. In previous pathological studies, the controls comprise people who have died suddenly with a high proportion of deaths from ischaemic heart disease {Mastaglia, 2002 #1351; Jellinger, 2003 #1252}. In contrast, our control group comprised brain donors who died from a variety of medical conditions, including malignancy and sepsis with a minority dying from vascular causes. Hence one would expect our control group to have a lower prevalence of CVD rather than the greater prevalence
observed. Moreover, in our study, there was no significant difference between the control and PD group in the proportion of patients dying from vascular disease. The control group in this study comprised a higher proportion of women than the PD group and was slightly older, although not significantly, a factor accounted for in the statistical analysis. The higher prevalence of vascular disease in males would suggest that the prevalence of vascular risk factors in the control group would be accentuated if more males were available for study.

Inconsistency in the literature in relation to the prevalence of CVD in PD may be due in part to differences in the methodology used to identify CVD {Nanhoe-Mahabier W, 2009 #1332}. Some pathological studies have not assessed subcortical CVD {Mastaglia, 2002 #1351} and our scoring system appears to have improved the sensitivity to detect the presence of SVD compared with other methods of grading vascular lesions {Jellinger, 2003 #1252}. A limitation of our study is that we only used routine haematoxylin and eosin stained sections so may have missed subtle pathological changes. Further studies are required to compare the methodology of assessing SVD with the aim of deriving a uniformly accepted and validated approach.

This is the first pathological study to include an analysis of vascular risk factors that associate with any group differences. We demonstrated a significant increase in the total number of vascular risk factors in the community dwelling control group compared with the PD group, consistent with our findings of more SVD in these subjects. Nevertheless, when the cases and controls were matched for age and number of vascular risk factors, the increased severity of SVD largely remained suggesting a lower prevalence of CVD in PD.

Of the individual vascular risk factors, heart disease was nearly three times more prevalent in the control group than the PD group. Significantly lower levels of lipids, total cholesterol and triglycerides and a lower incidence of diabetes have been described in a retrospective study comparing two groups of patients with PD, one treated with levodopa and one untreated {Scigliano, 2009 #830}, although in a large prospective population sample, high cholesterol level at baseline was associated with an increased risk of PD {Hu G, 2008 #1324}. We found a higher prevalence of smoking in the PD group (35%) compared with normal controls (22%) . As the number of smokers in our study is small and the difference between the two groups does not reach significance, we cannot draw any conclusions from this finding. Nevertheless, our findings contrast with the literature in which smoking appears to confer protection against PD with several epidemiological studies reporting a lower risk of PD amongst smokers {Hernán, 2002 #1340}. Although the mechanism of this association is not understood, the duration of smoking appears to be more important than smoking intensity {Chen H, 2010 #1328}. However, it remains unclear whether smoking is a specific risk factor for SVD {Sibon, 2004 #1323}.
Mindful that our study cannot identify cause and effect, we can only speculate on the relationship between reduced vascular risk factors and CVD in pathologically-confirmed patients with PD. A number of previous studies have suggested that levodopa therapy may reduce the risk of stroke in a variety of ways, depending on its intra-cerebral or systemic effects {Korten, 2001 #1354}. In a study of patients in a stroke registry, the prevalence of PD was significantly lower than expected in the general community and the authors postulate that this may be due to lower levels of intra-cerebral dopamine {Korten, 2001 #1354}. Levodopa is associated with decreased blood pressure in PD patients, possibly due to central and peripheral effects, which may reduce the risk of CVD {van Dijk, 1993 #563}; although some suggest an increased risk {Eigenbrodt, 2000 #574;Manolio, 1996 #575}. Conversely, immobility in PD may be associated with a higher risk of comorbid CVD {Nanhoe-Mahabier W, 2009 #1332}, although this may not have been so prevalent in the community dwelling PD cohort we have studied.

The present study has found no association between SVD and PD. Rather, we have demonstrated a lower prevalence of selected SVD pathological changes and associated vascular risk factors in the PD group compared with controls. Our findings contrast with the majority of previous neuropathological studies examining the relationship between CVD and PD. While the decreased SVD in this study may reflect the fewer vascular risk factor in the PD population, there may also be an underlying interaction between CVD and PD supporting the need for further research in this area.

Acknowledgements

Tissues were received from the Sydney Brain Bank, which is supported by Neuroscience Research Australia, the University of New South Wales and the National Health and Medical Research Council of Australia. The authors are grateful for the technical assistance of Mr Stephen Kum Jew, and to Ms Heidi Cartwright for assistance with the figure.

Author Roles

Research project: Conception – RS, JK, GH; Organization – RS, JK, GH; Execution – RS, JK
Statistical Analysis: Design – GH; Execution – RS, JK, GH; Review and Critique - DC
Manuscript Preparation: Writing of the first draft – RS; Review and Critique – JK, GH, DC

Full financial disclosure for the previous 12 months:

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**Consultancies**
- RS - none
- GH - none
- DC - none
- JK - none

**Advisory Boards**
- RS - none
- GH – Kolling Institute, University of Sydney & Centre for Brain and Mind Research, University of Newcastle
- DC - none
- JK - none

**Partnerships**
- RS - none
- GH - none
- DC - none
- JK - none

**Honoraria**
- RS - none
- DC - none
- JK - none

**Grants**
- RS - none
- GH - Funded as a Chief Investigator by National Health & Medical Research Council of Australia project grants #570850 (2009-2011), #1008307 (2011-2013), #1022325 (2011-2013), #1029538 (2012-2014) and fellowship grant #630434 (2010-2015), and National Health & Medical Research Council of Australia strategic research funds & Australian Research Council joint ageing well, ageing productively program grant #401162 (2007-2011). Received institutional support from the University of New South Wales in Infrastructure grants (2010 & 2011), Goldstar awards (2010, 2011 & 2012), and infrastructure support (2008-2015). Funded as Chief Investigator on research grants from Parkinson's NSW (2011), and Michael J Fox Foundation and Shake-it-up Australia (2012).
- DC - none
- JK – Project grant and Enabling (infrastructure) grant funding from the National Health and Medical Research Council of Australia.

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**Consultancies**
- Expert Testimony
- RS - none
- GH - none
- DC - none
- JK - none

**Employment**
- RS – Self employed; VMO St George Hospital
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- DC – Self Employed
- JK - University of Sydney (Professor of Neuropathology)

**Contracts**
- RS - none
- GH - none
- DC - none
- JK - none

**Royalties**
- RS - none
- DC - none
- JK – receives royalty from Henry Stewart publishing for contributions to a recorded lecturer series.

**Other**
- RS - none
- DC - none
- JK - none
Figure 1:

Photomicrographs demonstrating the grading scale used for the assessment of SVD. A-D gliosis; E-H vessel wall thickening; I-L perivascular dilatation; M-P pallor. Columns from left to right indicate nil; mild; moderate and severe pathology. Scale bar in D same for photographs A-L. Scale bar in M same for photographs M-P.