Association between peripheral neuroinflammation and DATSCAN data of the striatal nuclei in Parkinson Disease

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Abstract

Purpose: The etiopathology of Parkinson disease (PD) and several other neurodegenerative diseases is inseparable from inflammatory processes. Peripheral neuroinflammation has been postulated to be involved in the initiation and progression of PD.

Method: DATSCAN was applied to assess the dopaminergic loss in the brain of 391 PD patients and 151 healthy controls. Once the blood sample was collected, neutrophil to lymphocyte ratio (NLR) was calculated by autoanalyzer device on participants' whole blood samples.

Results: NLR was significantly correlated with caudal SBR in individuals with PD, even after adjustment for age and disease duration. NLR could not predict putaminal SBR in PD patients after adjusting for age or age and disease duration.

Conclusion: based on our findings, it is believed that peripheral inflammatory processes play a notable role in the pathogenesis of PD. NLR as an excellent representative for peripheral neuroinflammation could be considered a potential candidate biomarker for PD.

Keywords: peripheral neuroinflammation, PD, NLR, DATScan

1. Introduction

Parkinson disease (PD), a well-known chronic neurodegenerative disorder, is symptomatically typified with bradykinesia, impaired posture, rigidity, and tremor. It is now well-proved that the pathologic mainstay of PD is the demise of dopaminergic neurons located in the substantia nigra pars compacta (SNpc) (1-4). Previous studies have shown the presence of α-synuclein-containing deposits known as Lewy bodies in various areas of PD brains (5).

Lots of ergot mechanisms have been enumerated responsible for the neural loss in PD, including, but not limited to, Lewy body aggregation, iron overload, protein misprocessing, and oxidative stress (6). Among others, neuroinflammation, both cerebral and central, has been recently explained in the progression of PD (7).

Evidently, the brain is one of the immune-privileged organs. Notwithstanding, CD4+ and CD8+ T-lymphocytes have been reported to enter and invade the SNpc of idiopathic PD patients (8). Furthermore, it is documented that inflammatory cytokines, including TNF-α, can induce the activation of microglia and therefore the dopaminergic cell death. In this context, one of the valid indicators for peripheral inflammatory processes is the neutrophil to lymphocyte ratio (NLR) (9) representing the active inflammation (neutrophils) merged with immunological regulatory processes (lymphocytes). In this research, we studied the correlation between NLR values and DaTSCAN scores which signifies the dopaminergic activity.

2. Materials and methods

2.1 Participants

Participants involved in this research were recruited from Parkinson's Progression Markers Initiative (PPMI, http://www.ppmi-info.org/)(10). The study was approved by the institutional review board of all participating sites in the Europe, including Attikon University

Hospital (Greece), Hospital Clinic de Barcelona and Hospital Universitario Donostia (Spain), Innsbruck University (Austria), Paracelsus-Elena Clinic Kassel/University of Marburg (Germany), Imperial College London (UK), Pitié-Salpêtrière Hospital (France), University of Salerno (Italy), and in the USA, including Emory University, Johns Hopkins University, University of Alabama at Birmingham, PD and Movement Disorders Center of Boca Raton, Boston University, Northwestern University, University of Cincinnati, Cleveland Clinic Foundation, Baylor College of Medicine, Institute for Neurodegenerative Disorders, Columbia University Medical Center, Beth Israel Medical Center, University of Pennsylvania, Oregon Health & Science University, University of Rochester, University of California at San Diego, University of California, San Francisco. Written informed consent was obtained from all participants before study enrolment. The study was performed in accordance with relevant guidelines and regulations (10). These participants were tested and confirmed negative for any neurological disorders apart from PD. The participants' PD status was confirmed by Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) and the loss of dopaminergic neurons was observed DAT scans.

2.2 Blood sample collection

As it is pronounced in the PPMI biologics manual (http://www.ppmi-info.org/), baseline blood sample collection was completed at each participant site. PAXgene blood RNA tubes were utilized for the collection of blood samples following the study protocol. The number of neutrophils and lymphocytes were calculated by autoanalyzer device on participants’ whole blood samples, right after the collection.

2.3 Data Acquisition

Parkinson's Progression Markers Initiative (PPMI) database was the source of clinical, serological, and imaging data for this study (www.ppmiinfo.org/data) (10). All individuals with PD underwent DaTSCAN™ imaging at screening, months 12 (Visit 4), 24 (Visit 6), and 48 (visit 10) or at prematural withdrawal (if a scan was not completed in the last 12 months). We also obtained DaTSCAN™ imaging from healthy controls at screening. Subjects without evidence of dopamine deficiency (SWEDD) had DaTSCAN completed at month 24. As a preventive measure, all females with the potential of pregnancy was confirmed negative for urine pregnancy test prior to receiving the DaTSCAN™ injection. Radiopharmaceuticals were provided as a unit dose, and detailed specifics of ordering the DaTSCAN™ dose were discussed at the center visit. The selected dose was assayed in a 10 ml syringe filled to a standard volume of 6 ml. The syringe was reassayed so that we could avoid geometry effects on the determination of actual injected dose following injection of DaTSCAN™. All of these data were recorded on the PPMI Scan Information Source Document. Before the DaTSCAN™ injection, we pretreated the participants with saturated iodine solution (10 drops in water) or perchlorate (1000 mg). The target dose for subjects was 185 MBq or 5.0 mCi of DaTSCAN™. The dose range for injection was 111 to 185 MBq or 3.0 to 5.0 mCi of DaTSCAN™. Participants were imaged 4 + 0.5 hours later. We took all measures to maintain a consistent imaging time post injection of DaTSCAN™, since the expunging of Specifically-bound activity from striatal binding centers occurs gradually.

Based on the principals, raw projection data were obtained on a 128 x 128 matrix stepping each 3 degrees for a total of 120 (or 4 degrees for a total of 90) projections into two 20% symmetric photopeak windows centered on 159 KeV and 122 KeV with an overall scan duration of nearly 30 – 45 minutes. Just after the scanning, the acquisition parameters were recorded in the scan acquisition document.

2.4 Data Interpretation

In screening visit and visit 4 (SWEDDS only), DaTSCAN data was assessed and interpreted at the imaging core lab by professional nuclear medicine physicians. The interpretation of the images was based on the visual evaluation. We considered the interpretation of screening imaging as the final inclusion criteria for registration into this study.

3. Results

Baseline clinical characteristics and detailed demographic data of subjects of this study are depicted in table 1. Four hundred and forty-four subjects (185 females and 357 males) consisting of 391 PD patients (mean age= 61.1±11.3 years) and 151 healthy controls (mean age= 61.6±9.6 years) was investigated by means of DaTSCAN. In PD patients, mean disease duration was 6.5±6.4 months. The mean score for UPDRS 1 (non-motor symptoms), UPDRS2, and UPDRS3 (motor symptoms) was 5.5±4.05, 5.9±4.2, and 20.4±8.8, respectively. Regarding Hoehn and Yahr staging for motor severity, 167 patients were in stage 1, 222 patients in stage 2 and merely two patients were in stage 3.

In search of factor(s) with possible correlation with SBR of caudate or putamen and NLR, we performed a simple Pearson, or Spearman's rank-order for the H&Y stage, correlation analysis in control and PD group separately. Afterward, partial correlation of NLR and SBR in caudate and putamen regions was implemented with potential confounding factors as covariates. As shown in table 2, NLR was negatively predicted by age and had a negative correlation with caudate region SBR values only in drug-naïve early PD group. Older age was correlated with more DAT deficit in the normal group but not in PD group. Eventually, longer disease duration predicted lower SBR scores in the putamen of PD group. Education years did not predict any difference in SBR estimates in either of the groups. As expected, putaminal and caudal SBR were correlated with r-squared values predicting as high as 72% of the total variance (r2=0.722, p-value <0.001, and r2= 0.44, p-value<0.001 for control and PD group respectively).

Olfaction dysfunction in PD group was negatively correlated with Putaminal DAT scores. More severe motor symptoms (UPDRS 3) and higher H&Y stage were associated with more severe DAT deficit in SPECT imaging of PD patients.

Next, we performed partial correlation analysis between striatal SBR and NLR values in PD group, using age, sex, and disease duration in PD group, as covariates. NLR was weakly but significantly correlated with caudate SBR in patients with PD, which persisted after adjustment for age and disease duration (r2=-0.0104, p-value=0.04) [Data not shown]. NLR could not predict SBR of putamen in PD patients after adjusting for age or age and disease duration in PD group (r2=-0.087, p-value=0.087, and r2=-0.079, p-value=0.12 respectively), or SBR in neither of striatal regions after adjustment for age and sex in control groups (r2=-0.06, p-value=0.47 respectively, and r2=-0.058, p-value=0.48 respectively) [Data not shown].

4. Discussion

Our research is the first study to investigate the association between PD patients' caudal and putaminal SBR and NLR values. Our results indicated that caudate SBR in PD patients is significant, however feebly, correlated with NLR. It is of note that this correlation is in place even after we adjusted for age and disease duration.

Neuroinflammation in PD has received much attention in recent years. There are two types of inflammatory processes in PD; the (over)activation of microglia in CNS and the invasion of peripheral immune system components, which our study investigates the importance of the latter. In an intriguing study by E Akıl et al. (11), the association between NLR and IPD was assessed. They collated 51 IPD patients against 50 healthy individuals and eventually concluded that NLR is potentially a novel biomarker in PD. Previous studies have demonstrated that the balance in T-lymphocytes is disrupted in

PD. For instance, CD4+- CD8+ T-cell ratios are decreased in individuals with PD compared to healthy controls (12). Nonetheless, in a very new study, the authors believed that NLR should not be considered as sufficient indicator of peripheral inflammation processes in PD, for various reasons, among which is that NLR does not indicate the different subgroups of T lymphocytes.

As it is clear, the previous results are discrepant as some of our results in this study; we demonstrated that NLR is negatively correlated with caudate SBR in patients with PD. At the same time, our results showed that NLR is not predictive of putamen SBR in patients with PD after the adjustment for age or age and disease duration. Furthermore, none of caudate and putamen SBR was predicted by NLR in the healthy group. Microglial cells, when activated, increase their receptors and secrete different neurotoxins. It has been documented activated microglia are present in the substantia nigra and striatal nucleus in PD (13, 14). A number of pathways and mechanisms have been postulated regarding the dopaminergic depletion due to microglial activation such as nuclear factor kappa-light-chain-enhancer of B-lymphocytes (NF- JB), toll-like receptors (TLR), and cyclooxygenase 2 (Cox-2) (15). Some studies are in favor of this fact that peripheral inflammation responses might exacerbate the previously-existed neurodegeneration in the CNS (16, 17). However, weighing the importance of central inflammation against peripheral inflammation, the main credit for neurodegeneration is given to cerebral inflammation rather than peripheral inflammation.

From the first introduction of PD, numerous imaging methods have been established for the assessment of PD patients. DATSCAN is one of the most applicable imaging techniques for diagnosis of individuals with PD. Deficits in the DAT values is indicative of demise of dopaminergic cells in substantia nigra and striatum (18-20). Based on our results, there was a negative correlation between NLR and age in de novo PD patients. Regarding healthy controls, more severe deficits in DATSCAN was

associated with older age. In contrast, there was no such association within the PD group. In addition, we investigated the relation between duration of disease and SBR values in putamen and caudate of individuals with PD demonstrating that putamen SBR values are negatively correlated with duration of disease. An interesting related study reported that there is no significant difference between NLR values of idiopathic PD patients with disease duration less and more than five years (21). In this regard, another study revealed no association between disease duration and alternations in number of T-lymphocytes justifying this idea that peripheral inflammatory responses might linger all through disease duration (22). It also speculated that inflammatory-mediated dopaminergic loss might have been progressed long before the manifestation of PD symptoms (23).

5. Conclusion

In conclusion, we believe that peripheral inflammation, which in this research is represented by NLR is probably involved in the process of dopaminergic depletion in striatal regions. However, this relation between peripheral inflammation and Parkinson progression is not conclusive. Much more studies are required to further investigate this subject.

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Ethical approval: All procedures performed here, including human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Consent for publication

All authors consent for the publication of this study.

Availability of data and materials

All relevant data are available in the Parkinson's Progression Markers initiative (PPMI) database (http://www.ppmi-info.org/data).

Competing interests

The authors declare that they have no competing interests.

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Contribution

H.S.M, M.M.Z and M.H.A. contributed to the conception and design of the study; H.S.M and M.H.A. contributed to data collection and analysis; and H.S.M and M.H.A. contributed to writing the manuscript. F.G.S contributed to revising of the draft.

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Table 1. Between group demographics and baseline clinical characteristics of patients with Parkinson disease

Between Group Demographics	***PD patients (n=391)***	***Healthy controls (n=151)***	***P-Value*****	***Parkinson Disease Characterization***	***PD patients (n=391)***
Age (mean±sd)	61.1±11.3	61.6±9.6	0.582	**UPDRS I* (mean±sd)**	5.5±4.05
Female/Male no.	135/256	50/101	0.756	**UPDRS II* (mean±sd)**	5.9±4.2
Handedness (L/R)	42/349	31/120		**UPDRS III* (mean±sd)**	20.4±8.8
Education years (mean±sd)	15.5±2.9	16.1±2.9	**0.027**	**H&Y* stage (mean±sd)**	#167 stage 1 #222 stage 2 #2 stage 3
Neutrophil to lymphocyte ratio (NLR) (mean±sd)	2.5±0.94	2.2±0.84	**<0.001**	**GDS* score (mean±sd)**	#54 depressed (5≤ score) 2.31±2.4
Duration of disease in months (mean±sd)	6.5±6.4	-	-	**MOCA* score (mean±sd)**	27.1±2.3
Mean SBR* score in Caudate nucleus dominant affected side (mean±sd)	1.83±0.55	2.82±0.76	**<0.001**	**Adjusted UPSIT score (mean±sd)**	22.4±8.2
Mean SBR* score in Putamen nucleus dominant affected side (mean±sd)	0.69±0.27	2.01±0.66	**<0.001**	**Modified Schwab & England ADL* (mean Z-score)**	0.84±0.53
				SCOPA-AUT* (mean Z-score)	0.76±0.63

* Striatal binding ratios (SBR)= Ratio of Dopamine transporter (DAT) binding (in the affected hemisphere or mean DAT binding of both hemispheres) in caudate and putamen, compared to occipital lobe as reference region, UPDRS I= Unified Parkinson's Disease Rating Scale part I non-motor symptoms, UPDRS II= part II motor signs and UPDRS III= part III motor signs, GDS=Geriatric depression scale, MoCA= Montreal Cognitive Assessment, H & Y stage= Hoen and Yahr stage, Adjusted UPSIT score =University of Pennsylvania Smell Identification Test adjusted for age and sex, modified Schwab & England ADL= overall activities of daily living, and SCOPA-AUT= Scales for Outcomes in Parkinson's disease-Autonomic calculated Z-scores
** *p-value* of independent sample t-test analysis is reported for age, education years and neutrophil to lymphocyte ratio, p-value of Pearson's chi-square is reported for gender, handedness and motor-dominancy side

Table 2. Correlation coefficient values of striatal binding ratio in caudate and putamen areas with subjects age and years of education and motor and non-motor scores of PD patients.

		Age (years)	*Education Years*	*Caudate SBR*	*Putamen SBR*	*NLR*	*Disease Duration*	*UPDRS Part III*	*H&Y stage*	*MoCA score*	*UPSIT score*
Control group correlates	***Age (years)***			-0.08	-0.01	**0.138****		**0.196*****	**0.245*****	**-0.191****	**-0.327****
	Years of Education			-0.031	-0.057	0.003	-0.009	0.035	**0.101***	**0.109***	0.029
	Caudate SBR	**-0.248****	-0.071		**0.44*****	**-0.114***	-0.034	**-0.178*****	**-0.102***	-0.014	-0.041
	Putamen SBR	**-0.193***	-0.002	**0.722*****		-0.087	**-0.106***	**-0.152****	**-0.117**	0.012	**0.125***
	NLR	0.084	-0.129	-0.082	-0.077		*0.088*	**0.117***	0.094	-0.03	0.073

*1- * p-value<0.05, ** p-value <0.01, ***p-value< 0.001, dark grey cells denote pairs with no meaningful significance*

2- PD= Parkinson disease, NLR= neutrophil lymphocyte ratio, SBR=striatal binding ratio, H&Y stage= Hoehn and Yahr stage, MoCA= Montreal Cognitive Assessment, UPSIT score =University of Pennsylvania Smell Identification Test adjusted for age and sex, UPDRS Part III= Unified Parkinson's Disease Rating Scale part III motor signs. Cells in right and top part of the table (shaded light grey) show Pearson's correlation coefficient (r^2) value for PD patients.