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Short Communication

Salivary Alpha-Synuclein to Differentiate Parkinson's Disease from Drug Induced Parkinsonism and Controls Using ELISA

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Abstract

Background: Alpha-synuclein is a promising biomarker for Parkinson's disease (PD).

Objectives: To test the diagnostic accuracy of salivary alpha-synuclein concentration to distinguish PD from drug-induced parkinsonism (DIP) and controls.

Methods: Between March 2017 and February 2020, total and oligomeric alpha-synuclein concentration were measured in 31 PD and 26 DIP patients and 20 age- and sex-matched controls. Diagnoses of PD and DIP were based on DAT-SPECT results. Salivary oligomeric alpha-synuclein concentration was assessed by ELISA and total alpha-synuclein concentration by electrochemiluminescence-ELISA.

Results: Salivary oligomeric alpha-synuclein concentration was undetectable in the three groups and total alpha-synuclein concentration was not different between groups (p=0.350).

Conclusion: Quantification of salivary alpha-synuclein with ELISA does not seem to be accurate to distinguish PD from DIP and controls.

ABBREVIATIONS

DIP: Drug-Induced Parkinsonism; PD: Parkinson's Disease: RT-QuIC: Real-Time Quaking-Induced Conversion

INTRODUCTION

Accuracy of Parkinson's Disease (PD) diagnosis based on clinical assessment is about 90% [1]. Drug-induced parkinsonism (DIP) is the second cause of parkinsonian syndrome after PD [2]. The presence of akathisia, orofacial dyskinesia and symmetric distribution of akinetic-rigid syndrome on clinical examination favor DIP over PD diagnosis, with a relative low specificity [3]. DIP is secondary to dopamine reversible receptor blockade, whereas PD is caused by progressive non-reversible nigrostriatal neurodegeneration. In 15% of DIP, parkinsonism persists or progresses after dopamine receptor blocking agent (DRBA) withdrawal, suggesting underlying PD. The parkinsonian syndrome, revealed by these treatments is not always taken into consideration although functional imagery could confirm the diagnosis of PD [4].

Alpha-synuclein is a promising biomarker for PD, as alpha-synuclein fibrils are major components of Lewy bodies and neurites. In PD, alpha-synuclein can aberrantly polymerize into oligomers, then fibrils with amyloid properties, leading to neuronal cell death. Braak and Beach [5] hypothesized the existence of an initial peripheral autonomic nervous system and anterior olfactory structures induction site of the misfolding of

the alpha-synuclein, whereby misfolded alpha-synuclein might spread from the lower brainstem through other regions of the central nervous system with a prion-like mechanism. Lewy body accumulation is also found in the superior salivary nucleus [6]. Therefore, alpha-synuclein may spread from neuronal salivary neurons to the epithelial cells of salivary glands. Alpha-synuclein has been observed on submandibular gland and minor salivary glands (even in early stages) biopsies of patients with PD but not in healthy subjects [7-8]. Previous studies have reported reduced concentration of total salivary alpha-synuclein and increased salivary oligomeric alpha-synuclein in PD patients compared with healthy subjects using ELISA [9-11] or electrochemiluminescence [12]. There are no data available on αsyn levels in DIP patients.

The aim of this study was to assess whether salivary total and oligomeric alpha-synuclein levels could be used to differentiate PD from DIP and controls.

MATERIALS AND METHODS

Patients

Consecutive early-stage patients with PD (n=31, Hoehn and Yahr ≤ 3 and disease duration ≤ 3 years) [13] and with DIP (n=26, age ≥ 45 years) and age- and sex-matched controls (n=20) had salivay analysis between March 2017 and February 2020, in the neurological units of Nîmes and Montpellier Hospitals and the psychiatric clinic of Quissac. Patients with atypical or vascular parkinsonism, familial history of PD, or oral cavity pathologies were excluded. Clinical diagnosis of PD or DIP was confirmed according to the blind interpretation of 123I-FP-CIT DAT-SPECT.

Controls were healthy volunteers without history or clinical signs of PD and patients with transient ischemic attack or minor stroke (National Institute of Health Stroke Score <3, without condition affecting salivary function), recruited from the Neurovascular Department.

For the PD and DIP patients, the Movement Disorder Society Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS III) [14] was used to assess motor signs. PD patients were evaluated under their usual dopaminergic treatment.

Drugs modifying salivary flow intake, history of diabetes mellitus, alcohol consumption and smoking were collected.

The PARKSYN trial (registered under NCT03156647), was a prospective multi-centric study, approved by the National Ethics Committee (CPP Sud Méditeranée III, Nîmes, France, ID-RCB: 2016-A01464-47). All subjects gave their informed consent for study participation and biological samples collection were stored at the Montpellier University Hospital certified NFS 96-900 biobank (Ref: BB-0033-00031).

Alpha-synuclein quantification

Salivary samples were collected from all subjects and analyzed at the laboratory of Biochemistry of the University Hospital of Montpellier.

After at least two hours of fasting, four hours without smoking and 12 hours without alcohol consumption, the odontologist completed an oral examination to exclude bleeding or infection.

Unstimulated saliva was collected after drinking a glass of still water, 15 minutes before collection, using two saliva absorber pads (SalivaBio Children's Swab (SCS), Sialimetrics device).

Saliva samples were immediately placed at 4°C to block proteolytic activity and centrifuged within 72 hours, at 1,300 xg at 4°C for five minutes, to remove residual particles. The supernatant was then aliquoted into 1 mL Eppendorf-type test tubes and stored at -80°C. Samples were analyzed in duplicate by a blinded independent biologist.

Total αsyn was assessed by electrochemiluminescence- ELISA (Meso Scale Discovery TM (MSD)), in samples of 60 µL (diluted 1/8). Oligomeric alpha-synuclein concentration was assessed by the human αsyn oligomer enzyme immunoassay ELISA Kit (MyBioSource, MBS730762, in samples of 50 µL (diluted 1/2). Standard curves and quality control samples gave satisfactory results.

Statistical Analysis

Statistical analysis was conducted using SAS (9.4; SAS Inc., Cary NC). Results were expressed in median (25–75 IQR) because of their distribution. The numbers and associated percentages were given for categorical variables. Alpha-syn concentrations were compared between the groups by a Kruskal-Wallis test. Other statistical comparisons between groups were performed by a Chi 2 test or by a Fisher test for the categorical variables and by a Wilcoxon-Mann-Whitney test for the continuous variables. Dosage reproducibility was assessed using the intraclass correlation coefficient (ICC; 95% CI). All statistical tests were conducted as 0.05 two-sided tests.

RESULTS AND DISCUSSION

Baseline and clinical characteristics were comparable between groups, except for more frequent symmetric parkinsonian symptoms (p=0.0014), orofacial dyskinesia (p=0.015) and more frequent smoking (p=0.0011), in the DIP group compared to PD group (Table 1).

Oligomeric αsyn concentrations were below the detection threshold (S1=100 pg/ml and S2=250 pg/ml) for 95% of the samples, preventing comparison of concentrations in the three groups.

No significant difference in the concentrations of total alpha-synuclein was observed between the three groups) (Figure 1, PD: 285.12 pg/ml [134.64; 731.2]; DIP: 323.44 pg/ml [161.3; 560.37]; controls: 437.5 pg/ml [254.95; 1176.85], p=0.3505).

Dosage reproducibility of total alpha-synuclein was satisfactory (ICC for total alpha-synuclein dosage of 0.83 [0.75;0.89] with a bias of -526 (4108) pg/ml).

In this study, total alpha-synuclein salivary levels did not

Table 1: Demographic and clinical features in PD, DIP groups and controls

Characteristics	Parkinson's Disease	Drug induced Parkinsonism	Controls	P value
Number of subjects	31	26	20	
Abnormal DAT SPECT	31 (100%)	0 (0%)	-	.
Age (years)	67 [61;73]	63 [55;71]	67 [56; 69]	0.437
Sex, male (%)	20 (65%)	16 (61%)	12 (60%)	0.943
Duration of parkinsonism (months)	3.3 [0.56;10.10]	1.9 [0.3;3.9]	-	0.632
Number of subjects				
with symmetric parkinsonism	5 (16%)	13 (50%)	-	**0.006**
with akathisia	0 (0%)	2 (8%)	-	0.204
with orofacial dyskinesia	2 (6%)	8 (31%)	-	**0.032**
Number of subjects with actual				
alcoholism	0 (0%)	2 (8%)	1 (5%)	0.341
smoking	3 (10%)	12 (46%)	1 (6%)	**<0.001**
diabetes mellitus	5 (17%)	4 (16%)	0 (0%)	0.200
drugs modifying salivary flow intake	7 (23%)	9 (35%)	6 (30%)	0.597
MDS-UPDRS III	27 [18;38]	31 [20;44]	-	0.418

Data are presented as median [IQR] or number (%). MDS-UPDRS III: Movement Disorder Society- Unified Parkinson's Disease Rating Scale part III; PD-NMS: Parkinson's Disease Non-Motor Scale.

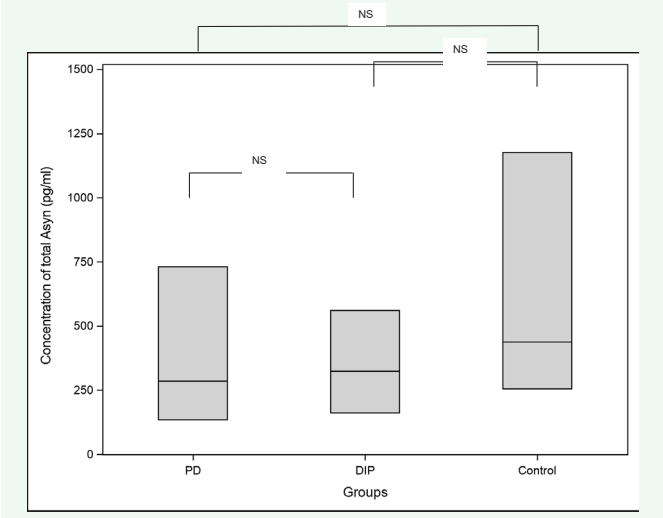

Figure 1 Data are expressed in median (25–75 IQR). Total salivary alpha synuclein measured in subjects with Drug Induced Parkinsonism (DIP), with Parkinson's Disease (PD) and controls.

allow the differential diagnosis between DIP, PD, and controls. Oligomeric alpha-synuclein salivary levels were undetectable.

Based on the very low concentration of total alpha-synuclein in the group of PD patients in the previous study (5.08 +/- 3.01 pg/ml) [10], we used an ultra-sensitive electrochemiluminescence-alpha-syn ELISA technique (Meso Scale Discovery TM (MSD)), validated for salivary analysis, instead of classical ELISA technique alone. As expected, using this method, total alpha-synuclein concentrations in our study were higher compared with the results of Vivacqua et al. study: a median of 285.12 pg/ml versus 5.08 ± 3.01 pg/ml. However, total alpha-synuclein salivary levels here were not different among groups and especially between PD and controls. This discrepancy can first be explained by the characteristics of our population. Recently diagnosed PD can be associated with lower salivary alpha-synuclein levels [7] but also with misdiagnosis (atypical parkinsonism) or with a different pathophysiology (genetic PD). Our control group was not validated by DAT-SPECT. Second, in the lack of consensus, our collection technique may have influenced the results [15]. Timing of sample, stimulation of saliva, sampling device, collected volume, protease inhibition, hemoglobin level assessment and centrifugation power differ between studies.

While we used the same technique as Vivacqua et al. to measure oligomeric alpha-synuclein concentration, levels were under the threshold of detection of the kit which questions the reliability of the technique in our population. Oligomeric anti-alpha-synuclein ELISA kit is not validated for saliva, and the specificity of the dosage can be reduced by an increased matrix-effect [16-17]

Another study [11] using ELISA found that oligo alpha-synuclein concentration was increased in the saliva of PD patients but not enough to discriminate patients from controls.

However, recent interesting results have been achieved with immunoprecipitation-based real-time quaking-induced conversion (IP/RT-QuIC) in serum of PD patients (AUC: 0.86 (95% CI 0.74–0.99) [18]. Salivary α-syn RT-QuIC possessed good diagnostic accuracy, with sensitivity of 83.78% (95% confidence interval [CI], 68.86–92.35), specificity of 82.61% (95% CI, 62.86–93.02), in saliva of de novo PD compared to controls [19]. Unfortunately, α-syn RT-QuIC has never been tested in DIP population.

CONCLUSION

Even if SPECT remains the best in vivo biomarker for differential diagnosis between PD and DIP, it is expansive and not always available suggesting that other methods are necessary. Alpha syn is a promising PD biomarker, RT-QuIC method could be useful to unmask idiopathic PD in patients with DIP.

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