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In search of Parkinson's disease biomarkers — is the answer in our mouths? A systematic review of the literature on salivary biomarkers of Parkinson's disease

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ABSTRACT

The identification of reliable biomarkers of Parkinson's disease (PD) is a pivotal step in the introduction of causal therapies. Saliva is a biofluid which may be involved in synuclein pathology in PD. We have reviewed current studies on salivary proteins and compounds in PD patients and healthy controls, and their potential application as biomarkers.

A systematic literature search of the Pubmed and Scopus databases was performed. A total of 198 studies were screened, of which 20 were included in our qualitative analysis. We conclude that the oligomeric form of salivary alpha synuclein is higher in PD patients, and that this may serve as a potential biomarker of PD. Salivary DJ-1 concentrations fail to differentiate PD patients from controls. Other enzymes and substances (heme oxygenase-1, nitric oxide, acetylcholinesterase) have been assessed in single studies. Salivary cortisol levels are higher in PD than in healthy subjects. Further validation of these findings is needed. Saliva may be a promising source of biomarkers in PD.

Key words: Parkinson's disease, saliva, biomarker, neurodegeneration

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Introduction

Numerous studies have been conducted with the aim of identifying potential biomarkers for Parkinson's disease (PD), the second most common neurodegenerative disorder [1]. Current research on causative treatment for PD is hindered by a lack of biomarkers and the recent introduction of potentially disease-modifying medications. The two most commonly assessed physiological fluids that are used for the detection of markers of PD are cerebrospinal fluid and blood. Acquiring samples of these two fluids causes at least some discomfort and pain to the patient. Saliva has advantages over these fluids: a relatively safe and less invasive collection process, and low levels of blood contamination, as well as many others.

Saliva production was initially investigated in PD with regard to drooling. It was already mentioned in the very first description of PD by James Parkinson in his "An Essay on the Shaking Palsy" [2]. Numerous studies were conducted to investigate the pathomechanism behind this phenomenon, revealing that: 1. Drooling is in fact caused by dysphagia rather than by higher-than-normal production of saliva in PD patients; and 2. Total saliva production in PD subjects may actually be lower than in healthy controls [3–7]. Some of these studies have indicated that saliva composition is different in PD patients than in healthy subjects. A meta-analysis by Masters et al. proved that total salivary protein concentration in PD patients is higher compared to controls [8]. Some authors have suggested that this may be caused by an over-secretion of proteins by the submandibular, sublingual and parotid glands [8, 9].

This interest in studies of saliva in PD is justified by recent histopathological discoveries, especially multiorgan involvement of PD-related α-synucleinopathy [10]. Submandibular and minor salivary glands have established involvement in α-synuclein pathology [11, 12]. Needle core biopsies of submandibular glands of PD patients have confirmed the presence of Lewy-type α-synucleinopathy in about 75% of cases, with even higher rates of positive findings in post-mortem histopathological studies [13–15].

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Therefore, saliva, as a product of these glands, may reflect their involvement in PD pathology. Especially unstimulated saliva seems to be of interest, as it is produced mainly by submandibular glands, as opposed to stimulated saliva which is produced by parotid glands.

The aim of this study was to summarise current findings on salivary biomarkers of PD, as well as to identify the most promising targets for future validation.

Methods

We searched the PubMed and Scopus databases using the key words 'Parkinson's disease', 'parkinsonism' and 'saliva' to identify publications in this area from 1 January 2000 up to 7 September 2019. The search was conducted in accordance with PRISMA guidelines for systematic review [16]. We included only studies in English. We assessed each of the 198 unique abstracts we found. We then considered only original publications pertaining to human saliva biochemical properties and saliva sample collection. We excluded review papers and meta-analyses. Additionally, we excluded from our systematic review studies assessing microbiome and oral hygiene of the mouth in PD, mechanisms of dysphagia, and treatment of hypersalivation and other clinical interventions, focusing solely on unique changes in saliva composition related to PD. We also excluded studies assessing only non-specific aspects of salivary composition in PD, such as electrolyte levels or total protein concentration. These restrictions reduced the number of reviewed publications to 20. The PRISMA flowchart of our selection process is presented in the section **supplementary material 1**.

To deal with the heterogenicity of findings, we further divided the studies into those assessing concentrations of α-synuclein, DJ-1, biomarkers of non-motor symptoms, and other salivary biomarkers.

Results

In total, we identified 20 studies on salivary biomarkers of PD, its progression or non-motor symptoms. Two papers report measurements of both salivary α-synuclein and DJ-1 [17, 18].

Salivary α -synuclein

α-synuclein is a presynaptic neuronal protein with robust expression in Lewy bodies [19-21]. Its detection is currently the gold standard in neuropathological confirmation of PD. It has also been extensively investigated as a potential biomarker.

We identified eight studies in which the authors measured levels of α-synuclein and its sub-forms in the saliva of PD patients and controls. A summary of the findings is presented in Table 1. Two studies originate from the same centre; however, the patients were recruited separately, and over different periods of time [22, 23]

Reports on concentrations of total α-synuclein in PD versus controls are inconsistent. In three papers, the authors report lower concentrations of total salivary α-synuclein in PD patients compared to controls [22–24]. In five other studies, no significant changes in total salivary α-synuclein were found [17, 18, 25–27]

In four studies including a total of 435 PD patients and 247 controls, the authors measured concentrations of the oligomeric form of α-synuclein separately. In all of these studies, higher levels of oligomeric α-synuclein were observed in the saliva of PD patients than in controls [22, 23, 26, 27].

One study also assessed concentrations of phosphorylated α-synuclein, but no difference was found between PD saliva and healthy controls [27].

Salivary DJ-1

The functions of the DJ-1 protein include acting as an antioxidant, transcriptional co-activator, and molecular chaperone. *DJ-1* mutations account for 1–2% of early onset cases of PD [28]. Concentrations of salivary DJ-1 in PD patients were assessed in four papers [17, 18, 29, 30]. These are summarised in Table 2. Although some authors reported higher concentrations of saliva DJ-1 in PD patients, these results were not confirmed after correction for total salivary protein concentration, which is also higher in PD patients [9, 29, 30]. A study by Stewart et al. assessed the liquid and cellular components of saliva (cheek epithelial cells) separately, but again no differences between PD patients and healthy controls were found [18].

Two studies reported higher DJ-1 levels in patients with more advanced PD. Kang et al. (2014) reported higher concentrations of DJ-1 in the saliva of PD patients with Hoehn and Yahr (H&Y) stage 4 compared to stages 1–3. However, no correlations with Unified Parkinson's Disease Rating Scale (UPDRS) scores, treatment or disease duration were found. They also observed a weak positive correlation between DJ-1 concentrations and putamen nucleus uptake of ^{99m}Tc-TRODAT-1 in PD patients [26]. Masters et al. reported that adjusted DJ-1 levels correlated positively with disease severity measured with the Movement Disorders Society-UPDRS ($p = 0.019$) [9].

Salivary biomarkers of non-motor symptoms

Findings on salivary biomarkers of non-motor symptoms in PD are summarised in Table 3.

Salivary cortisol levels were assessed in three studies as markers of non-motor symptoms: pain, depression and anxiety, and impulsive compulsive behaviours. Studies by Skogar et al. and Costa et al. reported significantly higher levels of total salivary cortisol in PD patients compared to healthy controls [31, 32]. Higher levels of cortisol correspond to PD patients with higher scores on the Hamilton Anxiety Rating Scale and the Hamilton Depression Rating Scale [32]. Pain and motor dysfunction does not affect cortisol levels in PD patients [31].

Table 1. Summary of studies assessing salivary α-synuclein levels in PD patients

Author	Sample size	Method of collection	Method of α-synuclein quantification	Total α-synuclein	Oligomeric α-synuclein	Additional findings
Cao *et al.* 2019	PD n = 74 HC n = 60	Unstimulated, saliva, drool into a 50 ml vial	ECL assay	No difference between PD and HCs	Higher in PD than in HCs (median 7.03 pg/ /ng *vs.* 0.92 pg/ng; p < 0.001)	Exosomes are present in saliva No difference in phosphorylated α-synuclein between PD and HC No significant correlation with H&Y stage
Vivacqua *et al.* 2019	PD n = 100 PSP n = 20 HC n = 80	Unstimulated saliva, drool into a 50 ml vial	ELISA	Lower in PD than HCs (7.104 ± 5.122 ρg/m *vs.* 28.444 ± 25.877 ρg/m; p < 0.05)	Higher in PD than in HCs (0.893 ± 1.949 ng/ /ml *vs.* 0.217 ± 0.191 ng/ml; p < 0.05)	Total α-synuclein in PSP higher than in PD
Vivacqua *et al.* 2016	PD n = 60 HC n = 40	Unstimulated saliva, drool into a 50 ml vial	ELISA	Lower in PD than HCs (5.08 ± 3.01ρg/ml *vs.* 31.3 ± 22.4 ρg/ml; p < 0.01)	Higher in PD than in HCs (1.062 ± 0.266 ng/ /ml *vs.* 0.498 ± 0.203 ng/ml; p < 0.01)	Mild positive correlation between total alpha-synuclein and disease duration, H&Y and MDS-UPDRS total score
Devic *et al.* 2011	PD n = 24 HC n = 25	Unstimulated saliva, drool into a 50 ml vial	Luminex assay	No difference between PD and HC	Not measured	Trend for α-synuclein to decrease and DJ-1 to increase in PD vs HC
Goldman *et al.* 2018	PD n = 22 HC n = 26	Not mentioned	ELISA	No difference between PD and HC	Not measured	
Stewart *et al.* 2014	PD n = 24 HC n = 198	Unstimulated saliva, drool into a 50 ml vial	Not specified	No difference between PD and HC	Not measured	DJ-1 and α-synuclein in the cellular component of saliva - assessed separately
Al-Nimer, Mshatat, and Abdulla 2014	PD n = 20 HC n = 20	Unstimulated saliva, details of collection not mentioned	ELISA	Lower in PD than in HC (65 ± 52.2 pg/ /ml *vs.* 314.01 ± 435.9 pg/ml; p < 0.02)	Not measured	
Kang *et al.* 2016	PD n = 201 HC n = 67	Unstimulated saliva, details of collection not mentioned	ELISA, HPLC	No difference between PD and HC	Higher in PD than in HCs (numbers not specified)	Significant differences in oligomer/total α-synuclein ratio in each H&Y stage

PD — Parkinson's disease, HC — healthy controls, PSP — progressive supranuclear palsy, H&Y — Hoehn and Yahr stage, UPDRS — Unified Parkinson's disease rating scale, ECL — electrochemiluminescence assay, ELISA— enzyme-linked immunosorbent assay, HPLC — high pressure liquid chromatography

In a study by Djamshidian et al., attenuated cortisol levels were also correlated with increased risk taking in PD patients with impulsive compulsive behaviours [33].

Saliva melatonin levels in PD and healthy subjects were assessed with regards to their role in circadian phase and habitual sleep onset time. No significant differences were found between unmedicated PD patients and healthy controls. Interestingly, the authors indicate the role of dopaminergic treatment in melatonin secretion, with the medicated PD group showing double the melatonin AUC of the unmedicated group [34].

One study identified reduced salivary substance P (SP) levels as an early biomarker of dysphagia in PD [35]. Saliva SP concentrations were significantly lower in PD patients with dysphagia compared to non-dysphagic patients (9.644 *vs.* 17.591 pg/mL; p = 0.001). The authors conclude that reduced SP levels may serve as an early dysphagia marker.

Other salivary compounds measured in PD

Fedorova et al. reported increased salivary acetylcholinesterase activity in PD. A total of 30 PD patients and 49 controls were involved. A colorimetric method was used to measure enzyme activity. Due to a significant overlap in results between PD and control groups, the authors declared it to be a marker of parasympathetic denervation rather that a biomarker of PD [36].

Table 2. Summary of studies assessing salivary DJ-1 levels in PD patients

Author	Sample size	Method of collection	Method of DJ-1 quantification	DJ-1 concentration	Additional findings
Devic *et al.* 2011	PD n = 24 HC n = 25	Unstimulated saliva, drool into a 50 ml vial	Luminex assay	No significant difference between PD and HC	DJ-1 concentration did not correlate with UPDRS score
Kang *et al.* 2014	PD = 285 HC = 91	Unstimulated saliva, drool into a 15 ml vial	Luminex assay	No significant difference between PD and HC	DJ-1 concentration did not correlate with UPDRS score; DJ-1 levels in H&Y-4 stage of PD was higher than those in H&Y 1–3 stage
Masters *et al.* 2015	PD n = 16 HC n = 22	Unstimulated saliva, drool into a 20 ml tube	Immunoblotting	Higher in PD than in HC (0.84 *vs.* 0.42 µg/ml, p = 0.001); after adjusting for total protein concentration no difference	Adjusted value for salivary DJ-1 concentration was positively correlated with UPDRS
Stewart *et al.* 2014	HC n = 198 PD n = 24	Unstimulated saliva, drool into a 50 ml vial, separation of cellular component of saliva	Not specified	No significant difference between PD and HC	

PD — Parkinson's disease, HC — healthy controls, H&Y — Hoehn and Yahr stage, UPDRS — Unified Parkinson's disease rating scale

A study by Huskić et al. analysed levels of salivary nitric oxide synthesis in the saliva of PD patients. A colorimetric method involving Griess reaction was used. The study included 16 PD patients and 16 healthy controls. The authors reported significantly lower salivary NO^2 concentrations in PD patients than in healthy subjects (5.02 ± 0.36 *vs.* 22.39 ± 1.24, p < 0.0001). The importance of this study is limited by numerous external factors that may influence oral nitric oxide synthesis [37].

Song et al. reported higher salivary concentrations of heme oxygenase-1 in a group of 58 PD patients versus 59 healthy controls (7.38 ± 0.95 *vs.* 4.87 ± 0.68; p = 0.03). ELISA and Western Blot methods were used. The authors noted higher salivary heme oxygenase-1 concentrations in patients with early idiopathic PD (Hoehn and Yahr stage 1–2) [38].

A study by Bermejo-Pareja et al. focused primarily on biomarkers of Alzheimer's disease. However, 51 PD patients and 56 elderly healthy subjects were involved as controls versus 70 Alzheimer's disease patients. Levels of salivary and plasma beta-amyloid were assessed with the ELISA method. The study revealed no significant difference in salivary beta-amyloid concentrations between PD and healthy subjects [39].

Chuang et al. reported higher methylation levels of DNA in PD. Saliva samples were collected from 128 PD patients and 131 controls. DNA methylation data was generated using the Illumina Infinium array. Saliva analysis identified five significant CpG sites [40]. The authors reported that gene/pathway enrichment analysis showed enrichment for gene sets related to neuron differentiation and projection.

Conclusions

Based on the available studies of the salivary proteins in PD, oligomeric α-synuclein is the most promising salivary biomarker of this disease. This is especially interesting when one considers that oligomeric forms of α-synuclein seem to be pivotal in the neurodegeneration process, mainly the formation of non-soluble deposits in PD [41]. α-synuclein oligomer levels have been described as being elevated in the cerebral cortex and brainstem of patients with idiopathic PD compared to age-matched healthy controls [42]. Increased concentrations of oligomeric forms of α-synuclein in cerebrospinal and lacrimal fluid have also been indicated as potential biomarkers of PD [43, 44].

Other proteins involved in the PD pathomechanism — total α-synuclein and DJ-1 — fail to differentiate between PD and healthy controls. Two other salivary compounds — heme oxygenase-1 and nitric oxide — have been reported by authors as potentially differentiating between PD and healthy controls. These findings come from single studies and need further validation. Substance P seems to be a potential early biomarker of dysphagia. This is especially interesting in the context of respiratory disorders (also due to aspiration) as the leading cause of death in PD patients [45].

The great limitation in salivary research is the variability of saliva composition. It has been proven that even the type of saliva collected (stimulated or unstimulated) may influence its microbiome composition [46]. Our review revealed discrepancies between concentrations of proteins reported by different groups. This is probably caused by different protocols for the collection and processing of saliva, and raises concerns about the reproducibility of some findings. Vivacqua et al. hypothesised that differences in reported salivary α-synuclein concentrations may be caused by different conformations of the protein, resulting in unpredictable antigen-antibody binding [23]. A study by Hong et al. also emphasised the importance of blood contamination of body fluids. Higher

Table 3. Summary of studies assessing salivary biomarkers of non-motor symptoms of PD

Author	Sample size	Non-motor symptoms assessed	Compound measured	Method	Results
Skogar *et al.* 2011	PD n = 59 HC n = 608	Pain	Cortisol	Spectria® Cortisol I^{125} radioimmunoassay	Morning cortisol concentration higher in PD than HC group Total cortisol secretion during day higher in PD than HC (AUC 112.8 nmolh *vs.* 81.1 nmolh) No significant correlations with age, gender, BMI, duration of PD, H&Y score, UPDRS III score, gait, pain and cortisol concentrations
Costa *et al.* 2019	PD n = 18 HC = 17	Anxiety, depression	Cortisol	ELISA	Higher in PD than HC (972.5pg/ml *vs.* 425 pg/ml, p = 0.03 Positive correlation between cortisol and HAM-A score (r = 0.46; p = 0.02), and HAM-D score (r = 0.09; p = 0.02)
Djamshidian *et al.* 2011	PD n = 28 (PD ICB+ n = 15, PD ICB- n = 13) HC n = 14	Impulsive compulsive behaviour	Cortisol	Immulite Immunoassay	Higher daily salivary cortisol levels in PD ICB- patients compared to healthy controls but no difference between PD ICB+ patients and controls.
Bolitho *et al.* 2014	HC n = 27 PD n = 29 (medicated PD n = 16; unmedicated PD n = 13)	Sleep cycle disturbances	Melatonin	Double-antibody radioimmunoassay	Unmedicated PD *vs.* HC — no difference in melatonin concentration Medicated PD group - greater AUC of melatonin compared to unmedicated PD (U = 31; P = .001) and HC (U = 87; P = .001), respectively
Muhle *et al.* 2018	PD n = 20 (PD with dysphagia n = 10; PD without dysphagia n = 10) No HC	Dysphagia	Substance P	ELISA	Saliva substance P concentrations significantly lower in PD with dysphagia than PD without dysphagia (9.644 *vs.* 17.591 pg/mL; p = 0.001)

PD — Parkinson's disease, HC — healthy controls, H&Y — Hoehn and Yahr stage, UPDRS — Unified Parkinson's disease rating scale, HAM-A — Hamilton Anxiety Rating Scale, HAM-D — Hamilton Depression Rating Scale

concentrations of both DJ-1 and α-synuclein may be falsely reported in contaminated samples [47]. Interestingly, while some studies did not observe a correlation of DJ-1 and α-synuclein with age, others have reported decreases in α-synuclein concentrations with advancing age [18, 26]. Sex may also influence salivary composition, as DJ-1 has been reported to be slightly higher in men, while α-synuclein was higher in women, in the study by Stewart et al. [18]. Another obstacle is the lack of a unified gold standard in terms of saliva collection protocol in biomarker research. Protocols differ in complexity. For some patients, collection may even cause distress and therefore influence salivary composition. Over 50% of patients with PD report hyposalivation and/or xerostomia, which may exclude them from research [48].

Future prospects in salivary biomarker research include much more robust identification of proteins and peptides. Large-scale studies of proteome are possible due to the availability of mass-spectrometry techniques, and these methods are becoming increasingly accurate with the implementation of Orbitrap spectrometers. Future studies on biomarkers in PD should lead to the unification and improvement of methods for studies on salivary biomarkers of PD-related neurodegeneration.

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