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Neuronal exosomes in saliva of Parkinson's disease patients: A pilot study

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Parkinson's disease (PD) is the most common neuro-degenerative movement disorder of old age affecting about 2–3% of population ≥ 65yrs of age worldwide [1]. But, there are still no reliable biomarkers as the molecular pathology markers like plasma or cerebrospinal fluid (CSF) α-synuclein has its oligomeric variants. These are based on the currently understood pathophysiology, individually neither correlate well with the disease severity nor discriminate between different PD with the sufficient confidence [2]. Recent findings of α-synuclein efflux and other proteins from CSF to peripheral blood incorporated into exosomes is a growing focus on exosomes as having important role in PD pathophysiology [3]. Furthermore, exosomes from PD patients have been shown to induce α-synuclein oligomerization, suggesting that pathogenic α-synuclein oligomers may be preferentially sorted into exosomes and act as a seed for fibril growth. Salivary glands release α-synuclein into the saliva, the mechanism yet-to-be defined, a fraction of this secreted protein may be linked to exosomes [4]. In our study, we have observed for the first time that there is increased level of neuronal salivary exosomes in PD patients than the healthy control. Further, we demonstrated a new method to correlate the concentration of total salivary exosomes with the PD patients.

Total 18 PD patients were recruited. All the PD patient's physical and neurological examinations were in accordance with the United Kingdom Parkinson's Disease Society Brain Bank criteria [5]. The inclusion criteria were used, the age of 40 yrs or older with 5 year disease duration and MMSE (Mini Mental State Examination) score > 23.4. Similarly, healthy controls (HCs) were also recruited with MMSE score of > 23. All the participants who suffered from diabetes mellitus, cardiovascular, cerebrovascular diseases, autoimmune diseases, hematological neoplasms and tumors or with a history of brain or gastrointestinal surgery were excluded. This study was approved by the ethics committee of AIIMS, India. The unstimulated saliva samples (5ml) were collected. The clarified saliva samples were mixed with PEG 6000, resulting in 14% final PEG concentration. The samples were incubated at 4 °C for 14 h, then spun down at 10,000 × *g* for 1 h. The each pellet was suspended in PBS buffer. The isolated salivary exosomes were checked for the RBC contamination by using antibody against hemoglobin (Fig. S1). Western blots were performed on the salivary exosomes. Further, a densitomeric semi-quantitative analysis was used to quantify the L1CAM and phospho α-synuclein bands intensity in blot images, using ImageJ software. Concentration of exosomes was measured by nanoparticle tracking analysis (NTA) at 1:1000 dilution in 1xPBS buffer. NTA is based on the Einstein Stokes equation; it helps to visualize as well as analyse (size, concentration) the particles in solution. Data analysis was carried out by using Graph Pad Prism 7 software and *P* values were calculated.

To rule out the age could be the factor for differences in the protein expression level between PD and HC, we performed Spearmen rank correlation between age, L1CAM and pospho α-synuclein level. No significant correlation was observed. Western blots analysis were performed to confirm and validate the presence of exosomes for CD63, L1CAM and phospho α-synuclein protein (Fig. 1 A, B and C). CD63 is the common marker for exosomes, whereas L1CAM is a marker for neuronal origin exosomes. Normalized protein abundance were calculated for both the L1CAM and phospho α-synuclein in the both PDs and HCs. Normalized Protein abundance for L1CAM in PD were observed 66.77 ± 21.90 in compare to HCs 29.80 ± 5.84 ($p < 0.0001$) (Fig. 1 D and E). Normalized abundance of phospho α-synuclein in PDs were

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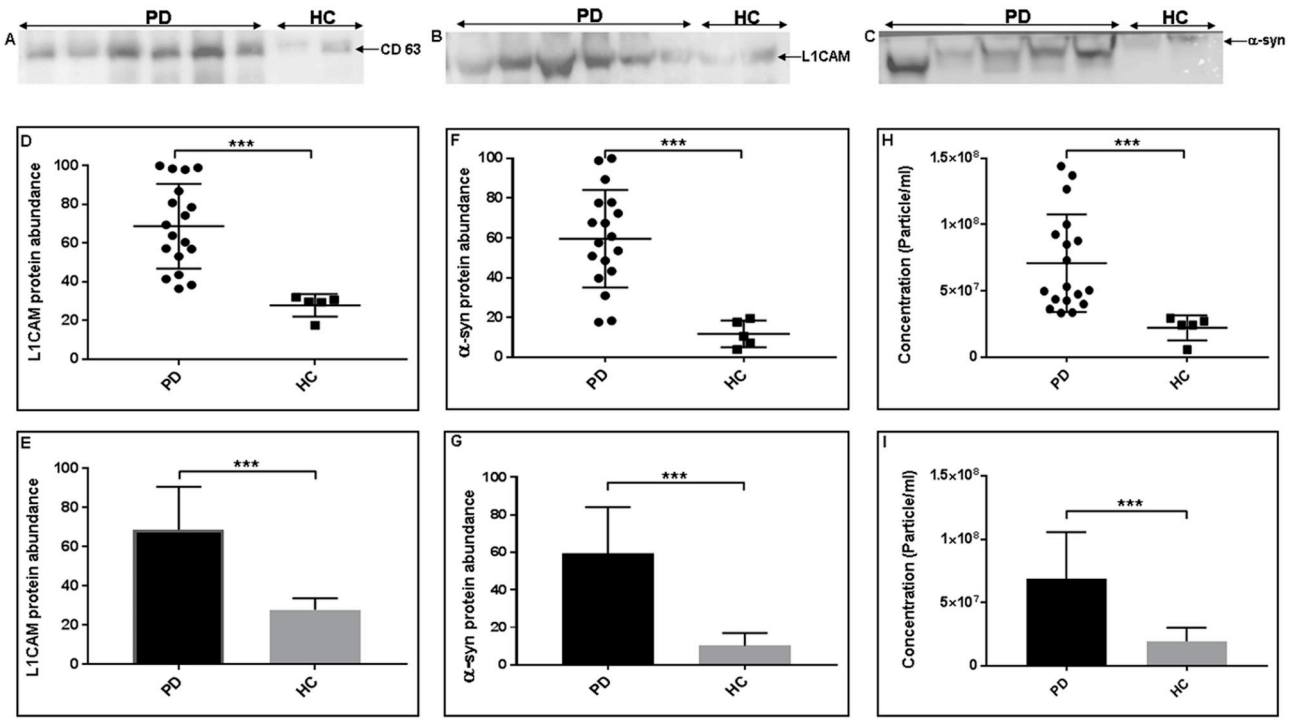

Fig. 1. A, B and C are Western blot profiling of CD63, L1CAM and α-synuclein protein expression from salivary exosomes of Parkinson' Disease (PD) and Healthy Control (HC). Densitometric analysis of L1CAM (D [Scatter plot] and E [box plot]) and α-synuclein (F [Scatter plot] and G [box plot]). The Western blot band intensities were measured using Image J software. Protein abundance was normalized for PD patients sample and healthy control sample, highest band intensity was considered equivalent to 100%. Comparision of L1CAM ($p = 0.0001$) and α-syn ($p = 0.0004$) protein level in salivary exosomes of PD and HC was done by unpaired *t*-test. H (scatter plot) and I (box plot) represent the Nanoparticle Tracking Analysis quantification of salivary exosomes from PD patients and healthy control ($p = 0.0001$). All Data expressed as median ± SD.

59.11 ± 24.51 in comparison to HC 10.43 ± 6.70 ($p < 0.0004$) (Fig. 1 F and G). To minimize the variation within the different blots, normalization was done to calculate protein intensities and converted into the percentage. We have calculated the ratio of phospho α-synuclein/total α-synuclein. This ratio was found to be significantly higher in PD patients as compared to the healthy control ($p = 0.003$) (Fig. S2). A novel method NTA showed higher concentrations of salivary exosomes in PD patients (1.44×10^8-3.33×10^7 particle/ml) then the HCs (2.94×10^7-5.72×10^6 particle/ml) (Fig. 1H and I) with p value 0.0001. To calculate ROC, the cutoff of the NTA method for exosomes concentration. A cutoff value of 3.1×10^7 particles/ml of salivary exosomes distinguishes PD patients from HC with 100% sensitivity and 100% specificity. Though this indicates an efficient detection method, further validation is required due to limited sample size. To correlate with the disease progression, we used the spearman rank correlation between phospho alpha synuclein and UPDRS ($r = 0.3326$, $p = 0.1641$). Due to the small sample size, correlation is not statistically significant, but it has shown a positive relationship between alpha synuclein and UPDRS.

The present study indicates salivary exosomal phospho α-syncluein levels are significantly higher in PD patients than HCs with the higher abundance of neuronal origin salivary exosomes in PD patients. This may indicate the increased salivary secretion of exosomes from neuronal endings in salivary glands in the PD patients. This is consistent with the pathophysiology of inter-neuronal spread of mis-folded α-syncluein fibrils via exosomes [1]. Our novel findings on salivary exosomes may prove to be a significant biomarker for PD detection at presymptomatic stage as well as an easy tool to objectively measure disease progression for drug efficacy studies.

Author contributions

KR, RM, SK, ES, VS, FN and SK contributed to the acquisition of the experiments and analysis of the data. AKD helped in NTA analysis and VG allowed to collect PD samples and critical analysis of the manuscript. PV and PB helped manuscript revision. KR, RM and SK contributed to conception, design and drafting the text.

Potential conflicts of interest

Nothing to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.09.008.

References

[1] W. Poewe, K. Seppi, C.M. Tanner, et al., Parkinson disease, Nat. Rev. Dis. Prim. 3 (2017) 17013.
[2] Y. Wang, M. Shi, K.A. Chung, et al., Phosphorylated α-synuclein in Parkinson's disease, Sci. Transl. Med. 4 (121) (2012) 121ra20.
[3] M. Shi, C. Liu, T.J. Cook, et al., Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease, Acta Neuropathol. 128 (5) (2014) 639–650.
[4] I. Devic, H. Hwang, J.S. Edgar, et al., Salivary α-synuclein and DJ-1: potential biomarkers for Parkinson's disease, Brain 134 (Pt 7) (2011) e178.
[5] L. Marsili, G. Rizzo, C. Colosimo, Diagnostic criteria for Parkinson's disease: from james Parkinson to the concept of prodromal disease, Front. Neurol. 9 (2018) 156.

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