Plasma Alpha Synuclein as a Potent Biomarker of Diseases with Synucleinopathies

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ABSTRACT

Objective: We explored whether plasma α-syn be used as a potential biomarker for synucleinopathies.

Materials and Methods: α-syn levels in plasma from 54 Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB) patents, 31 Alzheimer's disease dementia (AD), and 29 controls were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The mean age of the synucleinopathies group, the AD group, and the normal controls was 72.70, 74.26, and 62 years old. The median plasma α-syn levels in the synucleinopathies group, AD group and controls were 9.72 (4.41-25.30), 16.78 (7.68-51.41) and 16.65 (10.37-32.72) ng/ml, respectively (Independent-Samples Kruskal-Wallis test, $p = 0.026$). The α-syn levels in the synucleinopathies group were lower than those of AD and controls. There was a fair correlation between plasma α-syn levels and the sum of the Unified Parkinson's Disease Rating Scale (UPDRS) part 3 (spearman correlation coefficient $r = -0.261$, $p = 0.021$) but not with cognition measured by Thai Mental Status Examination (TMSE). The area under the receiver operating characteristic curve (ROC) was 0.710 between the PDD and DLB vs non synucleinopathies group (AD and normal controls) (SE = 0.052, $p \leq 0.001$). At the cut-off levels of 11.4 ng/ml indicated a sensitivity of 58% (95% CI 43.21-71.81%), specificity of 84.78% (95% CI 71.13-93.66%), positive predictive value (PPV) of 80.56%, a negative predictive value (NPV) of 65% and a precision of 70.83%.

Conclusion: The present results suggest that plasma α-syn could be a potential biomarker to differentiate synucleinopathies from Alzheimer's disease and the elderly with normal cognition.

Keywords: Alpha synuclein; biomarker; plasma; Parkinson's disease dementia; Lewy body dementia; Alzheimer's disease (Siriraj Med J 2023; 75: 864-870)

INTRODUCTION

Alpha-synuclein (α-synuclein) is a presynaptic neuronal protein that is neuropathologically related to synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA).[1,2] This protein had been explored as a diagnostic biomarker for synucleinopathies in cerebrospinal fluid (CSF), blood plasma, serum, and skin.[3-10] Many studies have shown that CSF alpha-synuclein can be used to differentiate between synucleinopathies and Alzheimer's disease, the most common neurodegenerative disease.[6,7,11-12] However, fewer studies have investigated alpha-synuclein in peripheral blood and its utility to differentiate between synucleinopathies and other diseases.[13-16] Furthermore, data on the plasma alpha-synuclein level in patients with PD are still inconclusive because previous studies had

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shown that blood alpha-synuclein level in patients with PD can be higher[7,17] or lower[18,19] compared to normal control subjects. Some studies have also found that there is no difference in the blood level of alpha-synuclein between PD patients and control.[13,20-22] Therefore, we investigated whether plasma alpha-synuclein levels can be used to differentiate between synucleinopathies, Alzheimer's disease, and control.

MATERIALS AND METHODS

Study design and population

The patients were recruited from the memory clinic at Siriraj Hospital, Mahidol University. The diagnosis of PD and Parkinson's disease dementia (PDD) was based on the clinical diagnostic criteria of the Movement Disorder Society for Parkinson's disease[23] and the recommendations of the MDS Task Force for the diagnosis of PDD.[24] Dementia with Lewy bodies (DLB) was diagnosed by using consensus criteria for clinical diagnosis developed by the DLB Consortium[25], and probable Alzheimer's disease (AD) was defined using the criteria of the National Institute on Aging and the Alzheimer's Association (NIA-AA).[26]

The sample size was calculated using the mean ± standard deviation (SD) from the reference literature.[27] Inclusion criteria were individuals whose age was more than 40 years, without a minimum year of education and diagnosed with Alzheimer's disease, synucleinopathies, or normal cognition. Exclusion criteria were individuals who were less than 40 years old or did not meet criteria for the diagnosis of AD or synucleinopathies. We also excluded patients currently using medications that can cause parkinsonism, such as antipsychotic medications. Acetylcholine esterase inhibitor and Parkinson medications (e.g., levodopa, dopamine agonist) were allowed. Therefore, we recruited 114 patients, consisting of 54 PDD and DLB patents, 31 AD patients, and 29 control participants.

We collected clinical information including age, sex, diagnosis, Thai version Mental Status Examination (TMSE) score, Addenbrooke's Cognitive Examination-Revised (ACE-R), Thai Activities of Daily Living Scale (ADL)[28], Unified Parkinson's Disease Rating Scale (UPDRS), and result of dopamine transporter scan (using ^{99m}Tc-TRODAT-1 SPECT image).

Blood sample and measurement of plasma alpha-synuclein concentration

Venous blood (5 ml) was drawn from the participants in the morning, 9.00 to 12.00 am, and the samples were processed within 30 minutes of collection. Plasma was prepared after collection of whole blood in an ethylenediaminetetraacetic acid treated tube. The processed samples were treated by centrifugation for 15 minutes at 1,500 g at room temperature. Following centrifugation, plasma was immediately transferred into clean and low residue polypropylene tube using a low residue tip. Plasma was stored at -80°C for less than 3 months prior to examination. No hemolyzed, icteric, or lipemic samples used. The levels of alpha-synuclein in the blood were tested by immunosorbent assay (ELISA) using the Human Phosphorylated Alpha Synuclein (PSNCA) ELISA Kit of MyBioSource, Inc, United States. The test was performed concurrently after 8-10 samples had been collected.

Statistics

For the statistical analyses, IBM SPSS Statistics 18 software was used. The blood α-synuclein data was not normally distributed and assessed by the Kolmogorov-Smirnov test. Mann-Whitney U was used to compare the results between two groups and the Kruskal-Wallis test was used to compare the results between more than two groups with adequate correction for multiple comparisons (Bonferroni). To analyze frequency difference of dichotomous variables, the chi-square test was used. The Spearman rank-order correlation coefficient was used to assess the correlations between variables. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to determine the cutoff value for α-synuclein.

RESULTS

Of the 114 participants in our study, there were 28 men (51.85%) in the synucleinopathies group, 15 men (45.45%) in the AD group and 3 men (11.11%) in the control group. The median age of all participants was 70.4 ± 10.463 years. Age frequencies showed median synucleinopathies (IQR) = 74 (66-77), AD = 75 (64-81), NC 61 (54.5-67.0); Independent-Samples Kruskal-Wallis Test $p<0.001$. The pairwise comparison of group diagnosis found that between synucleinopathies vs control: test statistic 32.987, standard error (SE) 7.604, standard deviation (SD) 4.338, $p<0.0001$; between AD vs control: test statistic 36.503, SE 8.533, SD 4.278, $p<0.0001$; between synucleinopathies vs AD: test statistic 3.516, SE 7.443, SD 0.472, $p=0.637$. The score of TMSE and ACE-R was lower in both disease groups compared to control and the ADL score in both disease groups was higher. The details of the score in each group are shown in Table 1.

TABLE 1. Patient's characteristics.

	Control	AD	Synucleinopathies (PDD and DLB)	
No patients	29	31	54	
Gender (Male, %)	3 (11.11%)	15 (45.45%)	28 (51.85%)	
Age (years)	61 (54.5-67.0)	75 (64-81)	74 (66-77)	p<0.001*
Education (years)	15.57 ± 6.53	10.35 ± 5.51	9.98 ± 6.09	0.07
TMSE	28.5 (27-30)	18 (14.5-23)	19.5 (13.25-24)	p<0.001*
ACE-R	90 (86.5-96)	37 (29-51.75)	40 (21.75-52)	p<0.001*
ADL score	0.54 ± 2.65	11.62 ± 7.66	10.20 ± 7.82	p<0.001*
UPDRS part 3	0	0 (0-1)	36 (20-44)	p<0.001¥

*p<0.001 between AD vs control, and between synucleinopathies vs control.
¥p<0.001 between synucleinopathies vs control, and synucleinopathies vs AD.
Data are presented as n (%), mean ± standard deviation, or median (interquartile range).
p-value corresponds to one-way and Kruskal-Wallis

For plasma alpha-synuclein level, median in synucleinopathies groups, AD groups, and controls was 9.72 (4.41-25.30), 16.78 (7.68-51.41), and 16.65 (10.37-32.72) ng/ml, respectively (Independent-Samples Kruskal-Wallis test, p = 0.026). Pairwise comparisons of group diagnosis in plasma α-syn levels revealed that between PDD and DLB vs control: test statistic -15.99, Standard Error (SE) 7.61, SD -2.10, p = 0.036; between PDD and DLB vs AD: test statistic 17.39, SE 7.45, SD 2.34, p = 0.02; and between controls vs AD: test statistic 1.407, SE 8.54, SD 0.17, p = 0.869. The plasma level of alpha-synuclein in the synucleinopathies group was significantly lower than in both the AD and the control group. We look for correlation between plasma alpha-synuclein and other factors, such as age, education, cognitive score (TMSE) and UPDRS score, but found a fair correlation only between plasma α-syn levels and the sum of UPDRS part 3 (spearman correlation coefficient r = -0.261, p = 0.021). Data on the correlation of other factors are shown in Table 2. The area under the receiver operating characteristic curve (ROC) was 0.710 between the PDD and DLB group vs. non-synucleinopathies (AD and normal controls)(SE = 0.052, p ≤ 0.001) as shown in Fig 1.

At the cutoff levels of 11.4 ng/ml indicated a sensitivity of 58% (95% CI 43.21-71.81%), specificity of 84.78% (95% CI 71.13-93.66%), positive predictive value (PPV) of 80.56%, a negative predictive value (NPV) of 65% and a precision of 70.83%.

In our study, 15 participants were scanned using ^{99m}Tc-TRODAT-1 SPECT imaging. Eleven participants had a positive scan, five of whom were in the synucleinopathies group and six were in the AD group. Comparing the positive and negative groups in age, blood alpha-synuclein, UPDRS part 3, or cognitive score, did not show any significant differences.

DISCUSSION

In synucleinopathies (PD, DLB and MSA), we diagnosed mainly by clinical criteria while neuroimaging biomarkers, structural and functional, were used only to support and exclude other possible causes.[1,29] Previous studies have shown the potential of cerebrospinal fluid (CSF) alpha-synuclein to differentiate synucleinopathies

TABLE 2. Spearman's rank correlation coefficient between each factor.

Variables	Correlation coefficient	Alphasynuclein (ng/mL)	Age	Education	TMSE	UPDRS
Alphasynuclein (ng/mL)	Spearman's Rho[a]	1				
	p-value	NA				
Age	Spearman's Rho[a]	-0.018	1			
	p-value	0.848	NA			
Education	Spearman's Rho[a]	-0.110	-0.275*	1		
	p-value	0.321	0.011	NA		
TMSE	Spearman's Rho[a]	-0.040	-0.293**	0.230*	1	
	p-value	0.686	0.003	0.038	NA	
UPDRS	Spearman's Rho[a]	-0.261*	0.235	-0.122	-0.460**	1
	p-value	0.021	0.039	0.379	<0.001	NA

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

[a] Spearman's rank correlation coefficient

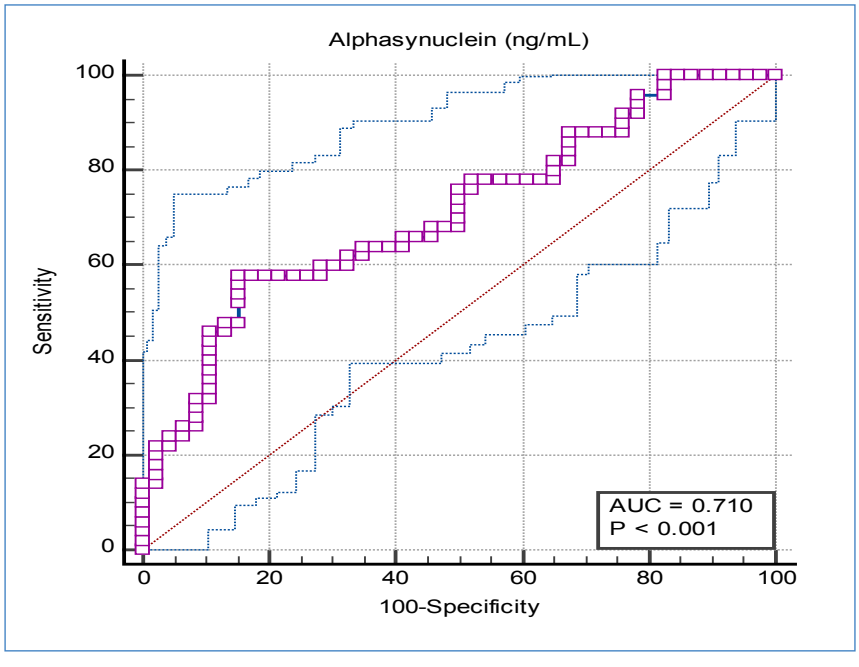


Fig 1. Receiver operating characteristic (ROC) curve for plasma α-synuclein levels to detect synucleinopathies (PDD and DLB). ROC curve of plasma α-synuclein levels for distinguishing synucleinopathies group from non-synucleinopathies group. AUC, area under ROC curve. Cut-off value = 11.4 ng/ml

from AD or normal cognitive control.[12, 30-31] However, due to difficult, invasive and costly of CSF collection compared to blood draws, so blood-based biomarkers are promising methods to use in clinical practice for the evaluation of neurodegenerative disease.[32-34]

Plasma alpha-synuclein in a previous study shows that it can be used to differentiate PD from AD or normal control[13-16], and most of the study found that plasma level of alpha-synuclein is higher than AD or control.[7,16-17] But in our study, plasma level of alpha-synuclein in synucleinopathies was lower than AD or control, and there are some other studies that reported similar results.[18-19] These conflicting results could be due to the different assay or method used to measure plasma alpha-synuclein[35] or because the main source of alpha-synuclein is red blood cells (RBC), so even low RBC contamination could affect the results.[36] Therefore, the handling method and the preparation of the sample are other factors that cause

inconsistency in the plasma alpha-synuclein level. In addition, disease severity and disease duration, which are different in each study, can also affect the results.[13]

According to published data, commonly used technologies for evaluation included the bead-based multiplexed immunoassay system (Luminex), sandwiched ELISA or ImmunoMagnetic Reduction (IMR).[16] If we look studies that use the same technique that we used[7,13,20-21,37-38], sandwiched ELISA, most had either higher or equal of plasma alpha-synuclein in the disease group compare to the control. It may be that, first, our studies inclusion criteria were synucleinopathies disease group, not just Parkinson's disease, which is different from previous research. Synucleinopathies consist of Parkinson's disease, Dementia with Lewy Body, and Multisystem Atrophy, which each of them had clinically and pathologically heterogeneous. For example, pathological hallmark of MSA was glial cytoplasmic inclusions (GCIs) predominantly in striatum, midbrain, pons, medulla, and cerebellum whereas for DLB, pathological hallmark was widespread of Lewy bodies or Lewy neurites in cerebral cortex and limbic system.[39-41] Due to different pathological seeding locations of alpha synuclein, clinical manifestations, criteria diagnosis, and prognosis of disease also different and that's explained why cut off value of blood alpha synuclein in our study different diagnosis of patient from previous studies, show different results. Second, our recruiting population was in an earlier stage and less severe than others with respect to the UPDRS score. Normally, plasma alpha-synuclein levels will increase over time along with disease severity[13] so our studies that patient were still in early stage, which mean alpha synuclein still didn't spread that much in central nervous system, measurement of alpha synuclein will be lower compare to other studies.

Plasma alpha-synuclein alone may not be suitable to differentiate parkinsonism from other neurodegenerations or controls and may need other biomarkers to increase accuracy. There are many recent studies that use multiple biomarkers, in CSF or blood, to produce greater sensitivity, specificity, and precision to differentiate between parkinsonism and control.[42-48] The most recent published studies[48] found that using combination of α-synuclein, Aβ42, Aβ40, Aβ42/40, and NfL could achieve a best diagnostic value in differentiating parkinsonian syndrome from healthy control with AUC 0.98. In future research, we may need to use multiple biomarkers, including plasma alpha-synuclein, to discriminate parkinsonism from other neurodegenerative diseases.

For imaging modality, we know for a long time that brain perfusion single photon emission computed tomography (SPECT) can be used for aiding diagnosis of Alzheimer's disease in early stage.[49] Because of difference abnormal perfusion area in each type of dementia, brain SPECT can help in differential diagnosis of dementia.[50] More specific type of SPECT, ^{99m}Tc-TRODAT-1 SPECT (TRODAT), is dopamine transporter imaging that can be used for differential diagnosis of Parkinsonism, between synucleinopathies and secondary Parkinsonisms.[51] It can also differentiate between Dementia with Lewy Bodies and Alzheimer's disease, which scan should be positive if patient had DLB.[52] We tried to analyze data between the positive and negative TRODAT SPECT group or between the positive TRODAT SPECT group in synucleinopathies and AD groups, but small sample sizes prevented us from detecting significance differences when comparing between them.

CONCLUSION

Plasma α-synuclein is a new biomarker for the diagnosis of Parkinson's disease and other synucleinopathies that may be useful to distinguish them from other diseases. Our study showed that the plasma level of α-synuclein is lower in synucleinopathies compared to Alzheimer's disease and normal control participants. At the cutoff levels of 11.4 ng/ml indicated a sensitivity of 58% (95% CI 43.21-71.81%), specificity of 84.78% (95% CI 71.13-93.66%), positive predictive value (PPV) of 80.56%, a negative predictive value (NPV) of 65% and a precision of 70.83%. The area under ROC was 0.710 between the PDD and DLB vs. the group without synucleinopathies (AD and normal controls) (SE = 0.052, $p \leq 0.001$). Plasma α-synuclein level correlates well with the motor sign of parkinsonism, measured by the sum score of UPDRS part 3, but not with cognition, evaluated using the TMSE score. Using multiple biomarkers, both fluid and imaging, will give more benefit to differentiate synucleinopathies from Alzheimer's disease.

Statement of Ethics

The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA no. Si 667/2017). Written informed consent was obtained from all participants in this study. All authors confirm that the research was conducted in accordance with the Declaration of Helsinki. Abstract and some content of this article was presented as Poster at Alzheimer's Association International Conference in 2020, Chicago but only abstract and figure was published online in supplementary issue of Alzheimer's & Dementia Journal. Content and figure in this article were all different from previous publication to avoid issue of plagiarism.

Conflict of interest statement

All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

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This was an unfunded study.

Author contributions

CD was involved in data and statistical analysis, interpretation of data, and manuscript writing. LW was involved in taking blood samples and processing them. SU was involved in data and statistical analysis. CR and PS participated in data collection. VS participated in design of study, interpretation of data, and manuscript revision. All authors approved the protocol.

Data availability statement

All data generated for this study are included in the article. There are no other datasets generated during the current study.

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