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Research article

Detection of α-synuclein oligomers in red blood cells as a potential biomarker of Parkinson's disease

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HIGHLIGHTS

- We showed that the ratio of α-syn oligomer/total RBC protein was higher in PD patients than in controls.
- There was no correlation between RBC α-syn oligomer levels and age at onset or UPDRS score in PD patients.
- The present results suggest that the RBC α-syn oligomer/total protein ratio can be a potential diagnostic biomarker for PD.

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterized by intracellular α-synuclein (α-syn) deposition. Alterations in α-syn levels in cerebrospinal fluid (CSF) and plasma of PD patients have been thought to be potential PD biomarkers; however, contamination arising from hemolysis often influences the accuracy of detecting α-syn levels in the CSF and plasma. In this study, α-syn oligomer levels in red blood cells (RBCs) obtained from 100 PD patients, 22 MSA patients, and 102 control subjects were measured by enzyme-linked immunosorbent assay. We showed that the ratio of α-syn oligomer/total RBC protein was higher in PD patients than in controls (29.0 ± 19.8 ng/mg vs. 15.4 ± 7.4 ng/mg, $P < 0.001$). The area under the receiver operating characteristic curve (AUC) indicated a sensitivity of 79.0%, specificity of 64.7% and a positive predictive value of 68.7%, with an AUC of 0.76 for increased α-syn oligomer/total RBC protein ratio. However, there was no correlation between RBC α-syn oligomer levels and age at onset, disease duration, age, UPDRS motor scale score or progression of motor degeneration in PD patients. The ratio of RBC α-syn oligomer/total protein was also higher in MSA patients than in controls (22.9 ± 13.9 ng/mg vs. 15.4 ± 7.4 ng/mg, $P < 0.001$). However, no significant difference was found for α-syn oligomer/total protein ratio between PD and MSA (29.0 ± 19.8 ng/mg vs. 22.9 ± 13.9 ng/mg, $P > 0.05$). The present results suggest that the RBC α-syn oligomer/total protein ratio can be a potential diagnostic biomarker for PD.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder whose diagnosis relies on cardinal motor symptoms, including rest tremor, rigidity, bradykinesia, and gesture instability [1,2]. However, the diagnosis based on motor symptoms has limitations for its inability to differentiate PD from other parkinsonian disorders due to overlapping symptoms and to detect the pathological change and progression of PD [3,4]. Therefore, identifying PD-specific biomarkers is critical for diagnosing PD [5] and tracking disease progression and response to treatment.

A body of evidence supports that α-synuclein (α-syn), a small presynaptic protein, plays a key role in the pathogenesis of PD [6–8]. Fibrillated α-syn has been identified as the major component of Lewy bodies and Lewy neurites, the pathological hallmarks of both sporadic and familial PD [9–11]. In addition, point mutations and multiplications in the α-syn gene have been linked to familial PD [12,13]. Moreover, polymorphisms of the α-syn promoter

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Table 1
Clinical data and RBC parameters of the study population [a].

Groups	Control (n = 102)	PD (n = 100)	MSA (n = 22)
Sex (male/female)	57/45	59/41	12/10
Age (years)	59.1 ± 11.7	55.0 ± 11.7	64.2 ± 7.8
Disease duration (years)	–	5.8 ± 4.2	4.1 ± 3.3
Age at onset	–	49.4 ± 12.2	60.3 ± 6.9[b]
UPDRS motor scale score	–	32.0 ± 13.1	–
Hoehn–Yahr stage (25th, 75th percentile)	–	2 (2, 2.5)	–
RBC α-syn oligomer to total protein (ng/mg)	15.4 ± 7.4	29.0 ± 19.8[c]	22.9 ± 13.9[c]

MSA: multiple system atrophy; PD: Parkinson's disease; RBC: red blood cell.
[a] Data represent mean ± SD.
[b] $P < 0.001$ vs. PD.
[c] $P < 0.001$ vs. control.

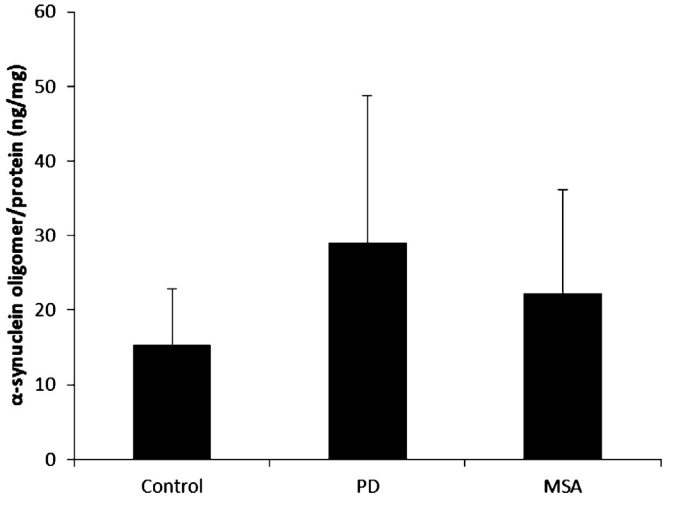

Fig. 1. α-Syn oligomer levels in RBCs from control subjects (n = 102) and patients with PD (n = 100) and MSA (n = 22), as detected by ELISA. Data represent mean ± SD of triplicate samples.

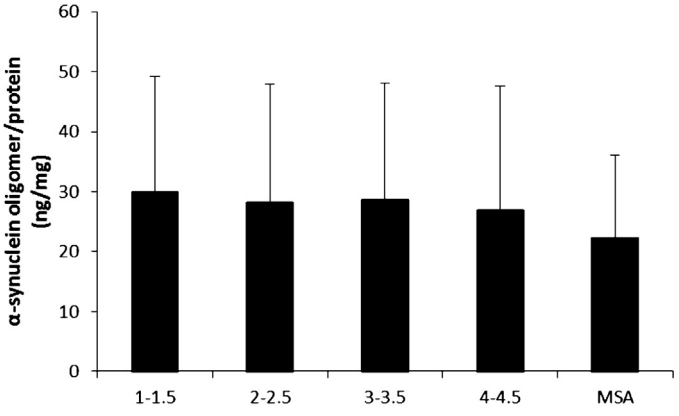

Fig. 2. RBC α-syn oligomer levels at different H–Y stages in PD patients and in MSA patients. Data represent mean ± SD.

have been associated to increased risk of PD onset [14]. Therefore, detection of α-syn alterations in CSF and plasma of PD patients is extensively accepted as an ideal biomarker for PD diagnosis and of disease severity. While some recent studies have shown that alterations in CSF α-syn concentration are promising biomarkers for PD diagnosis, efforts to detect blood α-syn have proved not successful due to the inconsistency of the results [15–17]. In both conditions, contamination arising from hemolysis is an inevitable problem that may preclude accurate assessments of α-syn alterations in CSF and plasma [18].

It has been reported that more than 99% of the α-syn in human blood resides in the red blood cells (RBCs) with less than 1% of the total detected in the plasma, platelets and peripheral blood mononuclear cells [19]. We therefore hypothesized that RBC α-syn level may serve as a potential peripheral biomarker for PD diagnosis or of PD severity. Since increasing evidence suggests that the oligomeric form of α-syn is neurotoxic that may trigger neurodegeneration [8], detection of oligomeric α-syn levels in RBCs are likely to have diagnostic potential.

2. Materials and methods

2.1. Participants

The study population consisted of 100 patients diagnosed with idiopathic PD, 102 healthy control subjects with no history of neurological illness, and 22 MSA patients. The number of each group reached the minimum sample size of 21 cases. All participants were provided with informed consent, and underwent an evaluation including medical history, physical and neurological examinations. Inclusion and exclusion criteria for PD and MSA were in accordance with those of the United Kingdom PD Society Brain Bank [20] and Quinn Criteria [21], respectively. Participant demographic information is listed in Table 1. The study protocol was approved by the Institutional Review Boards of Capital Medical University, Tiantan Hospital, Beijing, China.

2.2. RBC collection

Whole blood (5 ml) was collected from subjects in EDTA-coated tubes and aliquoted. RBCs were separated from plasma by centrifugation at 2200 × g and 4 °C for 20 min. Pellets were washed three times in Ca^{2+}-/Mg^{2+}-free phosphate-buffered saline (PBS) consisting of 137 mM phosphate buffer and 150 mM NaCl (pH 7.4) on ice and centrifuged at 2200 × g for 10 min. The supernatant was removed and the pellets were diluted 1:1 in ice-cold PBS and stored at −80 °C within 90 min after blood collection. Samples were thawed only at the time of analysis.

2.3. Measurement of protein concentration

Protein concentration in RBCs was measured using the bicinchoninic acid (BCA) protein assay kit (Sigma, St. Louis, MO, USA) at an absorbance of 405 nm relative to a protein standard.

2.4. Preparation of aggregated α-syn

Purified α-syn samples were solubilized with sterilized PBS to a final concentration of 100 μM. An Eppendorf tube containing 100 μl α-syn solutions was tightly sealed with Parafilm and incubated at