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REVIEW ARTICLE

Plasma alpha-synuclein levels in patients with Parkinson's disease: a systematic review and meta-analysis

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Abstract

Objective To date, there are no definitive biomarkers for diagnose Parkinson's disease (PD). The detection of α-synuclein (α-Syn) in plasma of PD patients has yielded promising but inconclusive results. To determine the performance of α-Syn as a diagnostic biomarker of PD, we used a meta-analysis.

Methods We identified 173 studies through a systematic literature review. From those, only studies reporting data on total α-Syn levels were included in the meta-analysis (10 publications, 1302 participants). Quality of studies was assessed by Newcastle-Ottawa scale.

Results The α-Syn levels were significantly higher in PD patients than healthy controls (standardized mean difference [SMD] = 0.778, 95% confidence interval = 0.284 to 1.272, $p = 0.002$). Similar results were found after omitting any individual study from meta-analysis, with SMD ranges from 0.318 (95% CI = 0.064 to 0.572, $p = 0.014$) to 0.914 (95% CI = 0.349 to 1.480, $p = 0.002$). According to meta-regression analysis, increased mean patients age (slope = − 0.232, 95% CI = − 0.456 to − 0.008, $p = 0.042$), increased total number of participants (slope = − 0.007, 95% CI = − 0.013 to − 0.0004, $p = 0.038$), and increased percentage of males (slope = − 6.444, 95% CI = − 10.841 to − 2.047, $p = 0.004$) were associated with decreased SMD of α-Syn levels across studies. We did not find any significant association between the SMD in α-Syn levels and disease duration, disease severity, and quality of studies. Most of studies applied ELISA assays.

Conclusion Total plasma α-Syn levels were higher in PD patients than controls. Analytical factors were important limitations.

Keywords Biomarker · Parkinson's disease (PD) · Meta-analysis · Meta-regression · Plasma · α-Synuclein

Abbreviations

α-Syn	α-Synuclein
CI	Confidence interval
CSF	Cerebrospinal fluid
ELISA	Enzyme-link immunosorbent assay
H&Y scale	Hoehn and Yahr scale
I^2	*I*-squared
NOS	New Castle-Ottawa scale
PD	Parkinson's disease
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
RBCs	Red blood cells
SMD	Standardized mean difference

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Background

Neurodegenerative diseases present a major problem for public health compromising the quality of life in today's aging population. Parkinson's disease (PD) affects 4.5 million worldwide, and it is predicted that this number will triple by 2030 [1]. Clinical diagnosis of PD is not always easy, and is only feasible when 60–80% of substantia nigra dopaminergic

neurons are already destroyed without clear diagnosis [2]. Moreover, currently available therapies are limited to stabilizing or ameliorating symptoms or slowing symptomatic progression, but without having a clear effect on the progression of neurodegenerative mechanisms. This fact highlights the need for the development of biological indicators to enable timely and accurate diagnosis, both in terms of daily practice and as regards the appropriate choice of patients for therapeutic protocols of drugs under development.

Alpha-synuclein (α-Syn) is a small, cytoplasmic 140-aa protein whose function remains ambiguous. It is observed in the presynaptic and nuclear compartments in healthy subjects but is also expressed in all tissues apart from liver. α-Syn in the cytoplasmic compartment reflects a pathological condition [3–5]. Cytoplasmic phosphorylated α-Syn aggregates, known as Lewy bodies, are pathological characteristics of synucleinopathies such as PD with or without dementia, and dementia with Lewy bodies. Exosomal release and transport of α-Syn was reported both in vitro and in vivo followed by intracellular uptake toxicity and cell apoptosis [6]. a-Syn oligomers, which are precursors to Lewy bodies, are also toxic to cells [7]. Hereditary types of PD are associated with mutations of the gene-encoding α-Syn [8]. Mice models with mutant α-Syn can result in a PD-like phenotype, the effects of which include nigra degeneration, kinetic symptoms, and response to levodopa therapy [9]. Such data point to α-Syn aggregates being involved in the breakthrough of PD. Thus, it is crucial to increase our knowledge since α-Syn may have an important role in the clinical diagnosis and treatment of PD.

As biomarker is defined, "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [10]. Given that abnormal a-Syn accumulation in peripheral fluids (cerebrospinal fluid (CSF), blood plasma, saliva) may reflect the abnormalities in the brain of PD patients, this protein has gained attention as such a surrogate biomarker for PD [11]. Compared to CSF, plasma is less costly and relatively non-invasive, easy-to-access biomarker. However, the determination of total α-Syn in plasma by ELISA and other similar techniques has yielded conflicting results in plasma levels of PD patients compared to those of controls. Total plasma a-Syn in PD patients was found to be higher [12, 13], lower [14, 15], or unchanged [16–19]. Moreover, oligomers [20] or phosphorylated forms gave also inconclusive outcomes [14, 17, 21]. Such discrepancies have often been attributed to pre-analytical and analytical confounders (diurnal variation, gender- or age-dependence, and importantly, blood contamination), different techniques (enzyme-link immunosorbent assay (ELISA), western blots, Luminex, mass spectrometry), and measurement of different α-Syn species (total vs oligomeric vs exosomal) in plasma.

The use of saliva to measure α-Syn is also an attractive possibility for biomarker assessment; its collection is easy and non-invasive and lacks possible blood contamination. However, compared with the plasma, the total protein content is much lower and protein concentrations may vary during the day. Due to the enrichment in protein material, α-Syn will have been sufficiently preserved in plasma (or serum) and plasma measurements will allow comparing the results between different studies. The levels of α-Syn are greatly affected by the biochemical environment of the protein including lipids (which are enriched in plasma) and proteolytic enzymes (which are present in saliva) [22, 23]. These two factors could significantly change the conformation status of α-Syn by either promoting the aggregation or the clearance of the protein. Of note, salivary total α-Syn levels could be manipulated by different α-Syn single nucleotide polymorphisms (SNPs) [24].

The objective of this study was to conduct a first-time systematic review of the literature and perform meta-analysis of the plasma α-Syn levels in PD patients as compared against healthy controls. Furthermore, this meta- analysis aimed to evaluate the diagnostic capacity of α-Syn for PD and explore the factors that influence α-Syn levels.

Methods

Search strategy and data extraction

We performed a systematic review and a meta-analysis, based on the PRISMA guidelines (preferred reporting items for systematic reviews and meta-analyses) (Fig. 1 and Supplemental Table S1) [25]. The MEDLINE database was used to seek related publications in the literature from 1970 to October 2018. The key words "Parkinson's disease, alpha synuclein/α-synuclein" was used for the initial search. A secondary search was then performed using the key words in conjunction with various combinations of the terms: "synucleinopathy/ies," "glia, neurons," "pathology," "pathogenesis," "parkinsonism," "plasma," "levels," "patients" biomarker. The bibliography listed for each article was also searched manually for any further related citations.

The inclusion criteria were (1) clinical diagnosis of PD based on accepted criteria [26] and (2) the relationship between levels of total α-Syn in plasma of PD patients as compared with healthy or neurological controls (i.e., without parkinsonian syndrome). Exclusion criteria were (1) the measuring of CSF, saliva, or other plasma species (oligomeric, phosphorylated, exosomal) of α-Syn in PD patients, (2) in vitro assessment of α-Syn aggregates, (3) animal models, and (4) relevant studies lacking numerical data.

Study selection and data extraction

Three investigators (AB, GP, KV) examined all titles and abstracts retrieved from the search. All full-text articles of identified

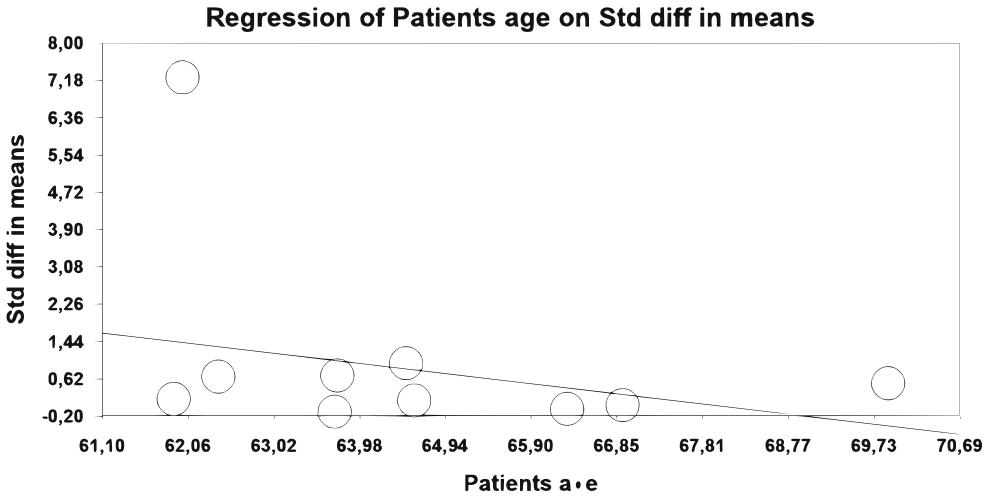

Fig. 1 PRISMA 2009 flow diagram

abstracts that met inclusion criteria were further scrutinized. In instances of debate during the eligibility assessment, another investigator (LS) reviewed the abstract/full text in question and made a final objective approval. In certain cases, the authors were asked to provide relevant data. If the same data set had been included in more than one article, only the most comprehensive study was included in the meta-analysis. The steps of the selection process were outlined in a PRISMA flow diagram (Fig. 1).

The variables were extracted from each manuscript by applying a structured template: the author's surname, year of publication, patient demographics (age, gender), type of assay used and the mean and standard deviation of disease duration, disease severity, and total plasma a-Syn concentration reported. The assessment of disease severity was based on the Hoehn and Yahr scale (H&Y scale) [27]. This scale ranges from 0 (no symptoms of PD), 1 (unilateral involvement), 2 (bilateral involvement impairment of balance), 2.5 (mild bilateral disease, with recovery on pull test), 3 (mild to moderate bilateral disease with some postural instability), 4 (severe disability but still able to walk), to 5 (confined to wheelchair or bed).

Quality assessment

Having retrieved the full text of studies that met the inclusion criteria, the three investigators proceeded independently assess the methodological quality of studies according to the New Castle-Ottawa scale (NOS) [28]. The NOS criteria contain (i) subject selection (scores, 0–4); (ii) comparability of subjects (scores, 0–2); and (iii) clinical outcomes (scores, 0–3). The NOS scores range between 0 and 9. A score ≥ 7 indicated that the study was of good quality (Table 1).

Statistical analysis

Our meta-analysis combined the data reported in individual studies in order to calculate effect size in means of standardized mean difference (means, standard deviations, and sample sizes). Standardized mean differences (SMD) and 95% confidence intervals (CI) in a-Syn levels between PD patients and controls were calculated. Given the high heterogeneity of studies, a random effects model (Der Simonian Laird method) and the generic inverse variance method were used. Statistical significance between patients and controls was assessed with two-tailed z-test. A p value < 0.05 was considered as statistically significant.

Heterogeneity was tested with Cochran's Q test, where $p < 0.10$ denotes statistically significant heterogeneity between studies and quantifies the degree of heterogeneity with the I-squared (I^2) index, which represents the percentage of the total variability across studies due to heterogeneity [29]. Increased value of I^2 index corresponds to increased heterogeneity, and in particular values of 25%, 50%, and 75% were assessed as low, moderate, and high heterogeneity, respectively [30].

To identify factors affecting the a-Syn levels, descriptive data were collected regarding the age of patients, age of controls, number of participants in a study, gender, disease duration, and disease severity. In addition, the quality of studies was assessed in accordance with the Newcastle-Ottawa scale and was used as a factor that may affect a-Syn levels. Subsequently, a meta-regression analyses (mixed effects regression, method of moments) was performed with the SMD between patients and controls as the dependent variable and the age of patients, age of controls, number of participants in a study, gender, disease duration, disease severity, and quality of studies as the independent variables (moderators). In this case, we calculated the value of slope and 95% confidence interval, while a p value < 0.05 was considered as statistically significant.

Sensitivity analysis was performed according to the "leave-one-out" method, i.e., removing one study each time and repeating the meta-analysis, in order to evaluate the impact of any individual study on the overall standardized mean difference.

Table 1 Quality assessment of included studies based on the Newcastle-Ottawa scale

Criteria	Lee 2006	Mata 2010	Shi 2010	Park 2011	Caranci2013	Foulds 2013	Wang 2014	Ding 2016	Lin 2018	Malec-Litwinowicz, 2018
Selection										
Representativeness of the sample	*	*	*	*	*	*	*	*	*	*
Sample size	*	*	*	*	*	*	*	*	*	*
Non-respondents										
Ascertainment of the exposure (risk factor)	**	**	**	**	*	*	*	**	*	*
Comparability										
Subjects in different outcome groups are comparable based on study design or analysis. Confounding factors are controlled.	*	**	**	*	**	**	*	**	**	*
Outcome										
Assessment of the outcome	**	**	**	**	**	**	**	**	**	**
Statistical test	*	*	*	*	*	*	*	*	*	*
Total	8	9	9	8	8	8	7	9	8	7

Newcastle-Ottawa Scale adapted for cross-sectional studies. Selection: (maximum 5 stars) (1) representativeness of the sample: (a) Truly representative of the average in the target population. * (all subjects or random sampling), (b) Somewhat representative of the average in the target population. * (non-random sampling), (c) Selected group of users, (d) no description of the sampling strategy. (2) Sample size: (a) justified and satisfactory. *, (b) Not justified. (3) Non-respondents: (a) comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. *, (b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory, (c) no description of the response rate or the characteristics of the responders and the non-responders. (4) Ascertainment of the exposure (risk factor): (a) validated measurement tool. **, (b) Non-validated measurement tool, but the tool is available or described. *, (c) No description of the measurement tool. Comparability: (maximum 2 stars) (1) the subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled: (a) the study controls for the most important factor (select one). *, (b) The study control for any additional factor. * Outcome: (maximum 3 stars) (1) assessment of the outcome: (a) independent blind assessment. **, (b) Record linkage. **, (c) Self report. *, (d) No description. (2) Statistical test: (a) the statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *, (b) The statistical test is not appropriate, not described or incomplete

Publication bias was assessed with Egger's weighted regression test (p value > 0.05 indicating no publication bias), Begg's funnel plot symmetry and Begg's and Mazumdar's rank correlation test (p value > 0.05 indicating no publication bias) [31]. Furthermore, the Rosenthal's fail-safe number ("file-drawer analysis") was calculated, which determines the number of additional unpublished studies that would be needed to turn a statistically significant overall effect size estimate (p value < 0.05) into a non-significant (p value > 0.05) [32]. In this case, an increased fail-safe number corresponds to lower publication bias. Finally, the "trim and fill" method was used to estimate the number of missing studies in our meta-analysis and to calculate a corrected overall standardized mean difference estimate [33].

Taking into consideration a medium effect size ($d = 0.5$), an average number of participants per group of 30 and anticipated high heterogeneity, at least 10 studies would be required to achieve a statistical power of 80% [34]. Moreover, according to Jackson and Turner [35], at least 5 studies are required to achieve power from random-effects meta-analysis that is greater than the studies that contribute to this. With regard to meta-regression analysis, only one independent variable (moderator) was used each time in accordance with the rule of thumb suggested by the literature for the modeling of conventional regression models requiring 10 studies for one moderator [36]. Comprehensive meta-analysis (CMA) software was used for the meta-analysis [37].

Results

From a total of 173 identified articles, 89 were excluded following title and abstract screening because they were not related to the subject, and 63 were excluded after full-text screening as they failed to fulfill the inclusion criteria (e.g., not English language, animals studies, reviews) (Fig. S1). No further relevant citations were found from weekly electronic database updates up to October 1, 2018. Only 10 of the 21 studies were included in the meta-analysis mainly due to incomplete numerical data [13, 16–19, 38–42] (Table 2).

The a-Syn levels were significantly higher in PD patients than healthy controls (SMD = 0.778, 95% CI = 0.284 to 1.272, $p = 0.002$) (Fig. 2).

Table 2 Characteristic of studies and outcomes included in the meta-analysis

Authors	Study type	Sample (W:M, age mean ± SD)DD	Method	Plasma α-Syn type: mean ± SD	Other measures/markers: mean ± SD	Main findings
Lee et al. 2006	Cross-sectional	**105PD** (60/45, 64.5 ± 11.4) DD**:** 44.6 ± 37.7 months **51 HC** (24/27, 62.8 ± 10.5)	ELISA	t-α-Syn PD: 79.9 ± 4 C: 76.1 ± 3.9	H&Y PD: 2.4 ± 3.9	↑PD vs C
Mata et al.2010	Cross-sectional	**86 PD** (20/66, 66.3 ± 9.4) DD**:** NA **78 HC** (44/34, 65.1 ± 10.3)	Luminex	t-α-Syn PD: 46.9 ± 32.6 C: 48.6 ± 39.9	NA	↔PD vs. C
Shi et al. 2010	Cross-sectional	**126PD** (33/93, 63.7 ± 10.6) DD**:** 8.3 ± 6.7 years **122 HC** (55/67, 58.9 ± 17.7)	Luminex	t-α-Syn PD: 36.8 ± 23.9 C: 39.5 ± 25.7	NA	trend↓PDvs C ↓age-dependent a-Syn in C
Park et al. 2011	Cross-sectional	**23PD** drug-naïve (12:11, 62.4 ± 12.7) DD**:** 2.2 ± 2.3 years **29NC*** (13:16, 60.1 ± 16.2)	ELISA	**t-α-Syn** PD: 0.16 ± 0.03 C: 0.15 ± 0.02	H&Y PD: 2.5	t-α-Syn: ↔ PD vs NC
Caranci et al. 2013	Cross-sectional	**69PD** (29/40, 64.59 ± 9.26) DD: 11.22 ± 4.46 years **110HC**(53/57, 64.31 ± 9.17)	ELISA	**t-α-Syn** PD: 10.55 ± 3.88 HC: 10.07 ± 3.16	H&Y PD: 9.63 ± 2,46	**t-α-Syn:** ↔ PD vs HC
Foulds et al.2013	Longitudinal	**189 PD** (70:119, 61.9 ± 9.7) DD: 5.10 ± 4.11 years **91 HC** (59:32, 65.3 ± 9.0)	ELISA	**t-α-Syn** PD: 1777.1 ± 3609.6 HC: 1221.5 ± 2233.1	NA	p-t-α-Syn:↑PD vs C **t-α-Syn:**↔ PD vs C ↔ t-α-Syn vs age, gender
Wang et al. 2014	Cross-sectional	**13 PD + FOG** (8/5, 66.92 ± 6.69) DD: 6.77 ± 3.77 years **15 HC** (9/6, 64.73 ± 4.88)	Luminex	**t-α-Syn** PD: 0.24 ± 0.3 HC: 0.23 ± 0.29	H&Y PD + FOG: 2.46 ± 0.78	↓PD + FOG vs HC
Ding et al. 2016	Cross-sectional	**37 TD-PD** (8/29, 63.73 ± 8.49) DD: 4.39 ± 3.75 years 26 HC (16/10, 64.77 ± 4.16)	ELISA	t-α-Syn TD-PD: 319.56 ± 64.22 C: 274.31 ± 70.71	H&Y TD-PD: 1.76 ± 0.75	↑PD vs C
Malec-Litwinowicz et al. 2018	Cross-sectional	39 PIGD (69.89 ± 10.36) DD: 10.22 ± 4.78ys 38 HC	ELISA	t-α-Syn PIGD: 4.93 ± 2.35 HC: 3.9 ± 1.56	H&Y PIGD: 2.51 ± 0.98	↔ AUC = 0.58; $p = 0.20$
Lin et al. 2018	Cross-sectional	37PD, (19/18, 62.0 ± 10.5) DD: 7:8 ± 5:2 years 35 HC (21/14, 62.6 ± 9.7)	Immunomagnetic reduction-based immunoassay	t-α-Syn PD: 0.99 ± 0.18 HC: 0.05 ± 0.02	H&Y PD: 2.3 ± 1.2	↑PD,APSvs HC, FTD-P

W women, *M* men, *SD* standard deviation, *DD* disease duration, *PD* Parkinson's disease, *PDD* Parkinson's diseases dementia, *PD + FOG* patients with PD with freezing of gait, *C* controls, *HC* healthy controls, *NC* neurological controls without parkinsonism*, *t-α-Syn* total α-Syn, ↔ without statistical significant differences among the study groups; correlation; *FOG-Q* freezing of gait questionnaire, *IMR* ultra-sensitive immunoassay utilizing immunomagnetic reduction, *H&Y* Hoehn and Yahr, *NA* not available

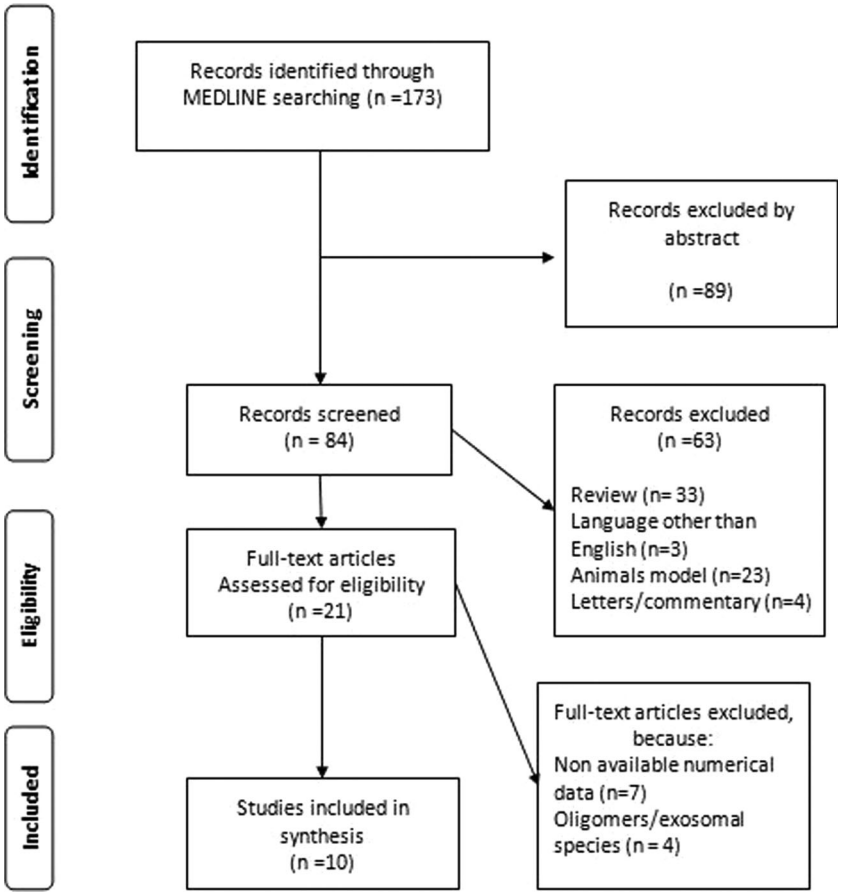

Fig. 2 Forest plot displaying standardized mean difference and 95% confidence intervals in a-synuclein levels between Parkinson patients and controls. Square dots show the effect size, horizontal lines through square dots show 95% confidence interval, and diamond shows the overall effect size

Results were robust in the sensitivity analysis, and their significance did not change after omitting any individual study from meta-analysis with SMD ranges from 0.318 (95% CI = 0.064 to 0.572, $p = 0.014$) to 0.914 (95% CI = 0.349 to 1.480, $p = 0.002$), (Fig. 3). An outlier value was identified with a standardized mean difference of 7.24, when standardized mean differences in other studies ranged from − 0.109 to 0.958. Even this outlier value did not alter the results of meta-analysis as confirmed by sensitivity analysis. Extreme low values in the control group were considered to be the main cause for the high standardized mean difference in the study of Lin et al. [40]. We found high heterogeneity across studies (Q [df = 9] = 151.3, $p < 0.001$; $I^2 = 94\%$).

Meta-regression analysis was performed to assess if the SMD in a-Syn levels between PD patients and healthy controls is associated with the age of patients, age of controls, number of participants in a study, gender, disease duration, disease severity, and quality of studies. Increased mean patient age (slope = − 0.232, 95% CI = − 0.456 to − 0.008, $p = 0.042$) (Fig. 4), increased total number of participants (slope = − 0.007, 95% CI = − 0.013 to − 0.0004, $p = 0.038$) (Supplementary Fig. S1) and increased percentage of male patients (slope = − 6.444, 95% CI = − 10.841 to − 2.047, $p = 0.004$) (Supplementary Fig. S2) were associated with decreased SMD of a-Syn levels across studies.

We did not find any significant association between the SMD in a-Syn levels and mean control age (slope = − 0.08, 95% CI = − 0.352 to 0.199, $p = 0.586$), percentage of healthy males (slope = − 5.133, 95% CI = − 13.232 to 2.967, $p = 0.214$), disease duration (slope = − 0.008, 95% CI = − 0.055 to 0.038, $p = 0.726$), disease severity (slope = − 0.191, 95% CI = − 0.542 to 0.163, $p = 0.288$), and quality of studies (slope = − 0.205, 95% CI = − 0.934 to 0.523, $p = 0.581$).

The funnel plot (Supplementary Fig. S3) suggested the publication bias was based on visual inspection asymmetry and this result was confirmed by Egger's weighted regression test (intercept = 7.581, standard error = 2.515, 95% CI = 1.794 to 13.368, t [8] = 3.02, $p = 0.017$) and Begg's and Mazumdar's rank correlation test (Kendall's tau = 0.49, $z = 0.489$, $p = 0.049$). Publication bias correction using the "trim and fill" method (random effects model) did not change the overall SMD estimate (corrected estimate = 0.778, 95% CI = 0.284 to 1.272) suggesting that no studies were missing. The Rosenthal's fail-safe number was 156, indicating that 156 additional studies would be needed to yield a non-significant result ($p > 0.05$) in our meta-analysis.

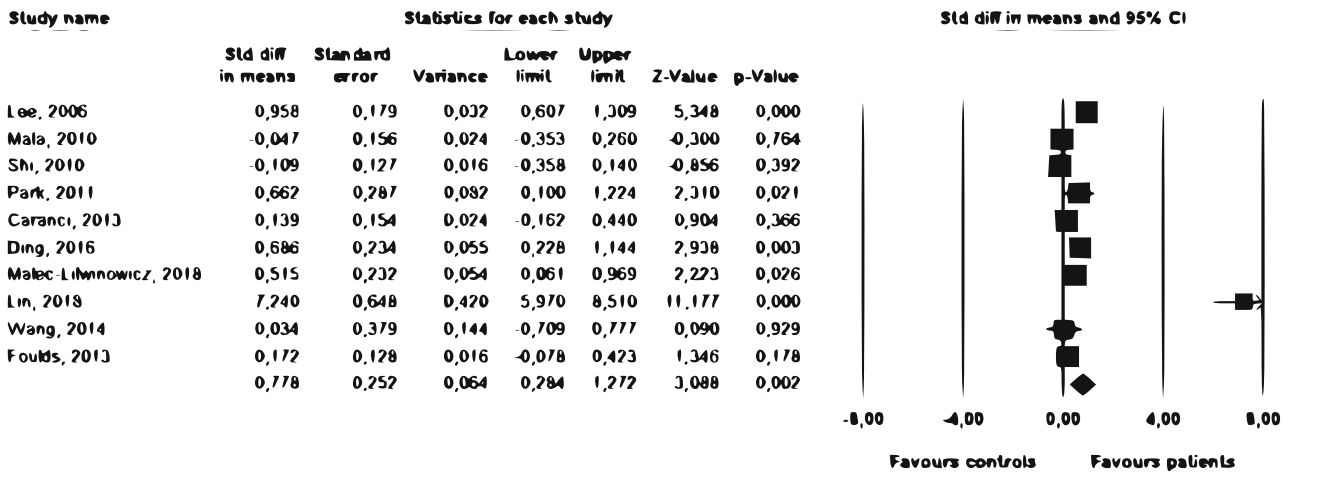

Fig. 3 Forest plot displaying sensitivity analysis with "leave-one-out" method. Square dots show the effect size, horizontal lines through square dots show 95% confidence interval, and diamond shows the overall effect size

Discussion

The meta-analysis revealed a higher concentration of total α-Syn in plasma of patients with PD was higher compared to that of controls and the SMD (between PD and controls in plasma α-Syn levels). These results did not change during sensitivity analysis, after omitting any individual study from the meta-analysis. Furthermore, meta-regression identified that increased patient age, total number of participants, and percentage of males were associated with decreased SMD of α-Syn levels. The physiological interpretation of α-Syn levels in the periphery has previously been debated in several studies; both the brain and the blood tissues are natural sources of α-Syn and a biological link between the two pools of the protein has not been established yet. As such, it is currently unknown whether defining the concentration of α-Syn in plasma reflects the actual levels of the brain-resident α-Syn. Nevertheless, increased plasma α-Syn levels in PD patients could indicate disease-related neuronal damage that leads to an elevation of extracellular α-Syn in the brain and subsequent leakage to the periphery. Alternatively, higher amount of total α-Syn in plasma could correspond to differences in the conformation properties of α-Syn in the patients compared to the control individuals. The conformation of α-Syn (monomers, tetramers, oligomers, aggregates) strongly depends on the biochemical composition of the biological sample which

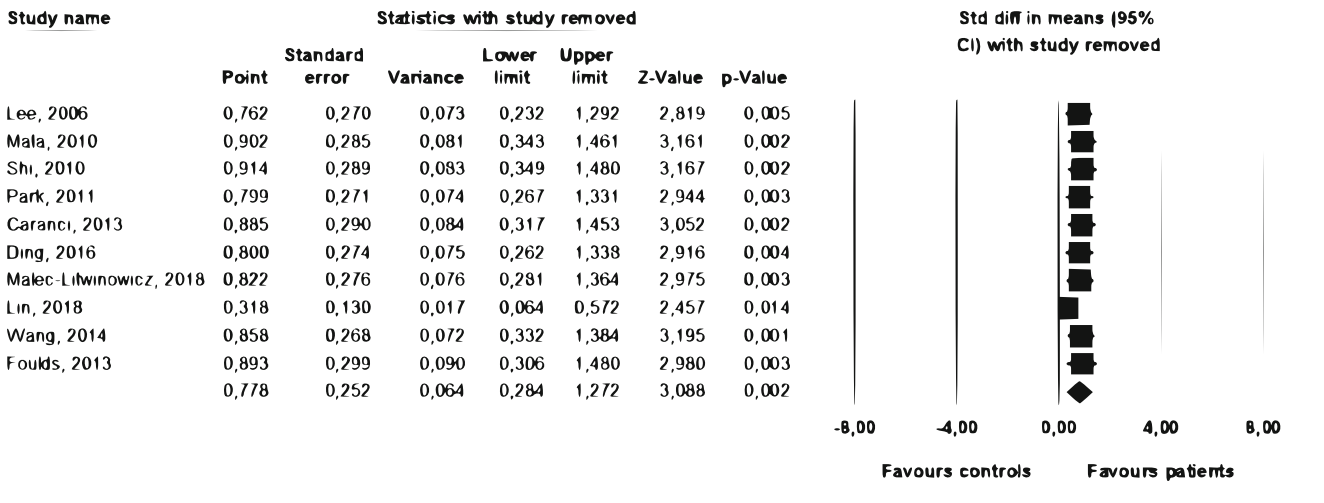

Fig. 4 Scatterplot displaying the negative relationship between patient age and SMD in a-synuclein levels between Parkinson patients and healthy controls. Circles show the studies and the line through the circles shows the negative relationship between patient age and SMD in a-synuclein levels. SMD standardized mean difference

can be different between the diseased and the healthy subjects. This could be particularly true when methods that rely on the conformation state of the protein, such as ELISA-based assays, are used to quantify α-Syn.

There was also a high heterogeneity across studies. This could be attributed to the co-existence of several components such as assays, disease staging, disease duration, and study setting. Most of studies included in our review applied either in-house or commercially available ELISA assays. Studies illustrated similar ELISA assay settings for total a-Syn. However, major differences were noted for (1) type of antibodies, (2) method of calibration curve, and (3) detection/quantification techniques [11]. Such differences may contribute to the high variability observed in mean values and SMD of plasma α-Syn.

Indeed, two studies with ELISA showed the total α-Syn levels in plasma of PD patients to be elevated [12, 13], in contrast to another study with western blot in which it was diminished [13]. Two other studies that measured plasma α-Syn via ELISA [18, 21] and mass spectrometry [18] found similar levels in both PD patients and controls. In larger cohorts that assessed levels of total a-Syn in plasma via Luminex assays, no difference was found between PD patients and controls [19]. Conversely, a recent study indicated that total α-Syn levels assessed using ELISA are decreased in patients with sporadic PD compared with controls, which was in line with findings in a previous report [14]. Notably, the small sample of this familial PD cohort could be attributed to the absence of significance.

An important issue that emerged from this review regards the high heterogeneity in results. The discrepancy of results could be explained by the fact that the principal origin of α-Syn are red blood cells (RBCs) (> 99% of its blood levels), with the residue in plasma [43]. Furthermore, platelet contamination in plasma, deficient age-matched controls, disparities in methods analysis, and sensitivity or specificity of antibodies may also affect plasma α-Syn level findings [42]. Confounders (hemolysis and platelet contamination) were not recorded in the majority of included studies.

Another interesting finding of this meta-analysis is the significant relationship between plasma α-Syn levels and male gender. In one meta-analysis, the incidence of PD was found to be 1.46 times (male-to-female ratio) greater in men of Western origin [44]. Moreover, plasma levels of α-Syn have been correlated with memory decline, delusions, and sleep problems in men with PD [16]. Intracellular α-Syn may be accumulated in men than in women. Estrogens are protective in women, by inhibiting α-Syn aggregation [16].

Although most of included studies were of acceptable quality, there were not without their limitations. First, there was an important inconsistency in the experimental protocol used between studies, particularly as concerns the type of assay used. Second, several studies were designed as cross-sectionals. Selection bias cannot be excluded owing of the inclusion of a group patient with diagnostic dilemma as well as with regard to disease stage. Most of the analyzed variables were not been separately examined in larger samples for total α-Syn. Consequently, specific recommendations cannot yet be formulated. Nevertheless, attention of the confounders that could modify the levels of plasma α-Syn is essential to future investigation on PD biomarkers. Results may be further influenced by other co-factors in the included studies, e.g., variability in the age of the patients, the severity of disease, and treatment types. Future studies should present mean and SD values of plasma α-Syn concentrations, sensitivity/specificity/ROC analysis, cut-off values, and histopathological examination.

This meta-analysis has its limitations. Firstly, we could only investigate total α-Syn type due to the paucity of studies for other species. Second, there was a lack of studies available on other parkinsonian syndromes for comparisons. Hence, our meta-analysis could only incorporate studies regarding PD and controls. Third, we only searched MEDLINE database. Although this accounts for an efficacious search strategy, a combination of other databases, (e.g., EMBASE, SCOPUS) and manual search may have identified more studies. Thus, the possible exclusion of other studies in the meta-analysis cannot be eliminated, which could signify publication bias. Indeed, the studies with ELISA showed the total α-Syn levels in plasma of PD patients to be elevated in contrast to other studies showing opposite trends. Interestingly, in larger cohorts that assessed levels of total a-Syn in plasma via Luminex assays, no difference was found between PD patients and controls. These results are limited to those particular setting. Future studies are needed with smaller heterogeneity to confirm these results.

However, the present meta-analysis also presents strong points: the literature was updated to comprise the latest evidence; limits of inclusion and exclusion criteria were defined for study selection; and similar outcomes were found after sensitivity analysis.

Conclusion

Total plasma α-Syn emerges as a new plausible biomarker for the diagnosis of PD that may prove useful in distinguishing between PD and controls, although its involvement in recording disease progression warrants further confirmation. Due to the limitations of the present meta-analysis, large longitudinal studies with standardized assays for plasma α-Syn are required to ratify the value of plasma α-Syn as a biomarker for PD.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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