

Exploring serum α -synuclein and its autoantibodies in essential tremor: implications for diagnosis and symptom correlations

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Abstract. There is no definite biomarker for confirming the diagnosis of essential tremor (ET) or differentiating it from other diseases, particularly Parkinson's disease. The present study aimed to investigate the serum levels of the α -synuclein protein (α -syn) and its autoantibodies in patients with ET compared with healthy controls and its relation to motor and non-motor symptoms in patients with ET. Serum α -syn and its autoantibodies were measured in 32 patients with ET and 32 age- and sex-matched controls. Both groups were assessed using the non-motor symptoms scale, MoCA, Beck Depression Inventory, Hamilton Anxiety Rating Scale, and the Short Form 36 Health Survey Questionnaire. Tremor was assessed using the Fahn-Tolosa-Marin Tremor Rating Scale. The serum α -syn concentration in patients with ET was significantly lower than that in healthy controls ($P < 0.001$), with a positive predictive value of 0.81 and a negative predictive value of 0.75, while the serum anti- α -syn autoantibody concentration was not significantly different between the two groups. There were no correlations between serum α -syn or its autoantibodies and patients' clinical characteristics. Furthermore, patients with ET had worse cognitive impairment, depression, anxiety, non-motor symptoms and quality of life. The serum α -syn concentration was lower in patients with ET than in controls, with favorable predictive values, suggesting that it could serve as a biomarker for ET diagnosis.

Introduction

Essential tremor (ET) is defined as a syndrome of isolated tremor of both upper limbs with a duration of at least 3 years, with or without tremor in other locations, such as the head, larynx (voice tremor), or lower limbs (1). Although the defining characteristic of ET has traditionally been its motor features, there is an increasing acknowledgment of additional NMSs associated with ET, such as cognitive decline, depression, anxiety and sleep disturbances (2-4). Currently, there is no definitive biomarker available for the diagnosis of ET. Therefore, the diagnosis relies heavily on the clinical presentation of the disorder (5).

α -Synuclein (α -syn) is a small protein that is encoded by the SNCA gene and is located on the long arm of chromosome 4 (6). The α -syn protein is considered a pivotal factor in the pathogenesis of a group of neurodegenerative conditions called synucleinopathies, which include Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy (7). Moreover, Lewy pathology has been detected in 25% of patients with ET, explaining the link between ET and PD (8).

Serum α -syn has been investigated and may serve as a potential biomarker for PD. However, investigating its level in patients with other parkinsonian syndromes and ET is important for confirming its value (9,10). Moreover, differentiating between ET and PD can be difficult, both in the early stages of these diseases and as they progress, since various types of tremors (such as rest, postural, kinetic and intention tremors) can be observed in both conditions. Therefore, investigating the α -syn protein in patients with ET could help differentiate between these two diseases (11). Interestingly, a recent study showed lower serum α -syn in ET and PD patients than in controls, with no difference between the two diseases (12).

The aim of the present study was to investigate the serum levels of the α -syn protein and its autoantibodies in patients with ET compared with those in healthy controls and its relation to tremor severity, non-motor symptoms (NMSs) and patient quality of life (QoL).

Materials and methods

In the present observational case-control study, patients were recruited from the outpatient movement disorders clinic at Ain

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Key words: essential tremor, α -synuclein, autoantibodies, nonmotor, quality of life

Shams University Hospital from December 2021 to May 2023 (Cairo, Egypt). The present study was approved by the ethics committee of the Ain Shams faculty of medicine (approval no. FMASU MS 700/2021), and written informed consent was obtained from all patients.

Sample size calculations. Using an online sample size calculator (<https://clincalc.com/stats/samplesize.aspx>); using two independent study groups design, with a dichotomous primary endpoint (diagnosis or no diagnosis), 15 subjects per group will provide a power of 80% with an alpha error rate of 0.05.

A total of 32 patients with ET and 32 sex- and age-matched healthy controls were included in the present study. Patients were diagnosed with ET by history and examination by movement disorder experts who met the diagnostic criteria for ET according to the Consensus Statement of the Movement Disorder Society on Tremor (1). The exclusion criteria included patients with a diagnosis of tremors of other etiology, for example, PD or dystonia; the presence of known causes of enhanced physiological tremor; concurrent or recent exposure to tremorgenic drugs, the presence of a drug withdrawal state, direct or indirect trauma to the nervous system within 3 months before the onset of tremor, a history or clinical evidence of psychogenic origins, mental retardation, and inability to perform the assessment. Matched healthy controls were chosen among subjects who showed no manifestations of ET or other neurodegenerative conditions, as excluded by a consulting neurologist.

Patients' tremors were assessed using the Fahn Tolosa Marin Tremor Rating Scale. NMSs, cognition, depression, anxiety, and QoL of patients and controls were evaluated using the non-motor symptoms scale (NMSS) (13), Montreal Cognitive Assessment (MoCA) - Arabic version (14), Beck Depression Inventory (BDI) - Arabic version (15), Hamilton Anxiety Rating Scale (HARS) - Arabic version (16), and the Short Form 36 Health Survey Questionnaire (SF-36) (17), respectively.

Other ancillary biochemical tests. It's important to note that while ancillary biochemical tests such as thyroid function tests can provide valuable information, they are not specific to ET and should be interpreted in conjunction with the clinical assessment and exclusion of other movement disorders. The final diagnosis of ET still heavily relies on the characteristic clinical features observed during examination and the absence of findings indicative of other neurological conditions. However, ancillary tests such as thyroid function tests can be useful in certain situations where there is a suspicion of secondary tremors due to thyroid dysfunction or other medical conditions that may mimic ET.

Laboratory testing of serum a-syn and its autoantibodies. Patients with ET and healthy controls who consented to participate in the present study underwent blood sample collection. Blood (3 ml) was withdrawn under sterile conditions and placed in serum separation tubes. The samples were then centrifuged at 3,000 x g for 15 min at 4°C, after which they were stored in -80°C freezers at Ain Shams Hospital, Ain Shams University (Cairo, Egypt).

ELISA tests were repeated in duplicates. The serum α -syn titers were expressed in picograms per milliliter (pg/ml) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit purchased from Elabscience Biotechnology, Inc. (cat. No. E-EL-H0983). The sensitivity of the assay was 9.38 pg/ml. Serum anti- α -syn autoantibodies were tested using a commercially available ELISA kit purchased from MyBioSource, Inc. (cat. No. MBS 2086950). The titers were estimated on the basis of a calibration curve of autoantibody standards and are expressed in nanograms per milliliter (ng/ml). The sensitivity of the assay was 0.1 ng/ml. The test steps for both kits were carried out according to the manufacturer's protocols.

Statistical analysis. The data were analyzed using the SPSS software package version 20.0 (IBM Corp.) and expressed as the mean \pm standard deviation. Statistical analysis was conducted using the unpaired Student's t-test, chi-square test and linear correlation coefficient. $P < 0.05$ was considered to indicate a statistically significant difference. Receiver operating characteristic (ROC) curve analysis was also used to determine the sensitivity and accuracy of the suggested biomarkers.

Results

The mean ages of patients with ET and controls were 51.75 ± 14.13 (32 to 77 years) and 51.34 ± 13.22 (32 to 72 years), respectively. In each group, there were 10 women (31.25%) and 22 men (68.75%). The two groups were age- and sex-matched. A total of 21 patients (65.63%) had a positive family history of ET. Additionally, there were 19 ET-plus patients in the ET group, representing 59.38% of the patients. The demographic data and clinical characteristics of the patients are described in Table I. Compared with controls, patients with ET showed significantly worse cognitive impairment according to the MoCA ($P = 0.001$), BDI, HARS ($P < 0.001$), NMSS total ($P < 0.001$) and subscale scores and all domains of the SF-36 ($P = 0.001$ or < 0.001) (Tables I and SI).

Correlations between NMS and QoL among patients with ET. The gastrointestinal and sexual domains were positively correlated with patient age ($P = 0.034$ and 0.040 , respectively), while the cardiovascular, perceptual problems/hallucinations, gastrointestinal and sexual functions domains were correlated with tremor severity ($P = 0.034, 0.026, 0.042$ and 0.012 , respectively). The NMSS total score was not correlated with the age of the patients, age of illness, illness duration or severity of tremor. The severity of tremor was negatively correlated with MoCA scores ($P < 0.001$) but not with depression or anxiety (Table II). Only the vitality domain was negatively correlated with the severity of tremor ($P = 0.017$). Role limitations due to physical health, vitality and bodily pain domains were positively correlated with the total MoCA score. All the QoL domains were negatively correlated with depression and anxiety, except for role limitations due to physical health and physical functioning domains, respectively. All SF-36 domains were negatively correlated with the total NMSS score, which means that increased severity of NMS is associated with greater impairment of QoL. All the SF-36 domains were negatively correlated with most of the NMSS domains (Table II).

Table I. Comparison of demographics, NMS and alpha-synuclein levels between patients with essential tremor and controls.

	Essential tremor group (n=32) Mean ± SD	Control group (n=32) Mean ± SD	T-TEST	
			T	P
Age (mean ± SD)	51.75±14.13	51.34±13.22	0.119	0.906
Sex (male/female)	22/10 (68.75/31.25%)	22/10 (68.75/31.25)	0	1
Family history (positive/negative)	21 (65.63)/11 (34.38%)			
Age of onset	39.94±11.87 (26-62)			
Duration of illness	11.59±5.78 (5-30)			
Phenotype: ET (ET plus)	13 (40.6%) [19 (59.4%)]			
FTMTRS Total	43.44±15.47 (28-82)			
Anatomic location	18.81±7.85 (10-41)			
Writing and pouring	12.38±4.17 (6-22)			
Functional disability	12.25±4.39 (6.0-24.0)			
Montreal Cognitive Assessment	25.34±2.80	27.34±1.66	-3.476	0.001
Beck Depression Inventory	19.63±7.42	8.50±1.78	8.251	<0.001
Hamilton Anxiety Rating Scale	18.09±8.35	8.00±1.92	6.666	<0.001
NMSS				
NMSS Total	48.84±23.94	10.94±3.09	8.892	<0.001
Cardiovascular	1.34±1.31	0.47±0.51	3.523	0.001
Sleep/fatigue	8.03±5.13	1.31±0.93	7.285	<0.001
Mood/cognition	12.88±8.05	3.31±1.12	6.655	<0.001
Perceptual problems/Hallucination	0.41±0.67	0.00±0.00	3.455	0.001
Attention/memory	11.38±6.07	1.53±0.62	9.129	<0.001
Gastrointestinal	1.88±1.68	0.88±0.71	3.103	0.003
Urinary symptoms	1.19±1.26	0.34±0.55	3.487	0.001
Sexual function	2.22±2.14	0.50±0.72	4.314	<0.001
Miscellaneous	9.28±4.71	2.59±1.07	7.838	<0.001
Serum biomarkers	no=28	no=26		
Serum a-syn (pg/ml)	275.38±155.36	425.55±80.44	-4.409	<0.001
Serum anti-a-syn autoantibodies (ng/ml)	115.12±128.22	110.55±44.76	0.172	0.864

SD, standard deviation; ET, essential tremor; FTMTRS, Fahn Tolosa Marin Tremor Rating Scale; NMSS, non-motor symptom scale.

Serum α -syn and anti- α -syn autoantibody levels in ET patients and controls. The serum α -syn concentration in patients with ET (28 patients, 275.36±155.36 pg/ml) was significantly lower than that in controls (26 subjects, 425.55±80.44 pg/ml) ($P<0.001$), while there was no significant difference in the serum anti- α -syn autoantibody concentration between patients with ET (115.12±128.22 ng/ml) and controls (110.55±44.76 ng/ml) ($P=0.864$) (Table I and Fig. 1). Correlation analysis revealed that serum α -syn and anti- α -syn antibody levels were not correlated with age, age of illness onset, illness duration or severity of tremor, NMSs or QoL (Table SII). There were no significant differences in serum α -syn or α -syn autoantibodies between patients with ET (12 patients) and patients with ET⁺ (16 patients) ($P=0.338$ and 0.574 , respectively) as shown in Fig. 2.

ROC curve analysis. Serum α -syn levels were able to discriminate between controls and patients with ET, with an area under the curve (AUC)=0.815 (95% CI=0.692-0.937), a cutoff a-syn level ≤ 354.08 pg/ml, a positive predictive value (PPV) of 0.81, and a negative predictive value (NPV) of 0.75

($P<0.001$). Serum anti- α -syn autoantibody levels were not able to discriminate between controls and patients with ET, with an AUC=0.632 (95% CI=0.463-0.801), a cutoff anti- α -syn autoantibody level ≤ 55.68 ng/ml, a PPV of 1, and an NPV of 0.65 ($P=0.126$) (Fig. 1). An analysis between the two subtypes of ET (ET and ET⁺) revealed no significant difference in the levels of serum α -syn or anti- α -syn autoantibodies with a P value of 0.8847 (Fig. 2).

Discussion

The present study explored serum α -syn and its autoantibodies in patients with ET and demonstrated lower values of serum α -syn among patients with ET than among controls, with a PPV of 0.81 and NPV of 0.75. Serum α -syn appears to differentiate between patients with ET and controls, with a cutoff value of 354.08 pg/ml. On the other hand, serum α -syn autoantibodies were not significantly different between the two groups involved in the present study. These findings are consistent with a study from Spain on 19 patients with ET,

Table II. Correlations between tremor severity and quality of life domains in patients with essential tremor.

		FTMTRS total	PF	RP	RE	VT	MH	SF	BP	GH
FTMTRS total	r		-0.326	-0.346	-0.309	-0.419	-0.114	-0.241	-0.18	-0.335
	P-value		0.069	0.052	0.085	0.017	0.533	0.183	0.324	0.061
Montreal Cognitive Assessment	r	-0.625	0.515	0.231	0.322	0.52	0.222	0.267	0.369	0.419
	P-value	<0.001	0.003	0.203	0.072	0.002	0.222	0.14	0.038	0.017
Beck Depression Inventory	r	0.341	-0.447	-0.336	-0.508	-0.672	-0.66	-0.584	-0.476	-0.544
	P-value	0.056	0.01	0.06	0.003	<0.001	<0.001	<0.001	0.006	0.001
Hamilton Anxiety Rating Scale	r	0.224	-0.206	-0.494	-0.532	-0.57	-0.72	-0.749	-0.75	-0.664
	P-value	0.217	0.258	0.004	0.002	0.001	<0.001	<0.001	<0.001	<0.001
NMSS										
Cardiovascular	r	0.376	-0.314	-0.644	-0.388	-0.367	-0.444	-0.518	-0.495	-0.514
	P-value	0.034	<0.080	<0.001	<0.028	<0.039	<0.011	0.002	0.004	0.003
Sleep/fatigue	r	0.17	-0.685	-0.335	-0.444	-0.589	-0.552	-0.419	-0.479	-0.498
	P-value	0.351	<0.001	0.06	0.011	<0.001	0.001	0.017	0.006	0.004
Mood/cognition	r	0.276	-0.447	-0.49	-0.55	-0.726	-0.822	-0.774	-0.78	-0.825
	P-value	0.127	0.01	0.004	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Perceptual problems/ Hallucinations	r	0.393	-0.073	-0.352	-0.123	-0.254	-0.066	-0.192	-0.186	-0.332
	P-value	0.026	0.691	0.048	0.504	0.161	0.72	0.292	0.309	0.063
Attention/memory	r	0.093	-0.286	-0.658	-0.524	-0.512	-0.57	-0.701	-0.588	-0.69
	P-value	0.614	0.113	<0.001	0.002	0.003	0.001	<0.001	<0.001	<0.001
Gastrointestinal	r	0.362	-0.489	-0.224	-0.254	-0.615	-0.536	-0.442	-0.655	-0.611
	P-value	0.042	0.005	0.217	0.161	<0.001	0.002	0.011	<0.001	<0.001
Urinary symptoms	r	0.232	-0.576	-0.04	-0.417	-0.604	0.533	-0.496	-0.514	-0.499
	P-value	0.202	0.001	0.827	0.017	<0.001	0.002	0.004	0.003	0.004
Sexual functions	r	0.437	-0.403	-0.374	-0.299	-0.619	-0.257	-0.422	-0.488	-0.515
	P-value	0.012	0.022	0.035	0.096	<0.001	0.155	0.016	0.01	0.003
Miscellaneous	r	0.129	-0.243	-0.57	-0.512	-0.627	-0.727	-0.757	-0.805	-0.783
	P-value	0.482	0.179	0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
NMSS Total	r	0.28	-0.536	-0.602	-0.6	-0.771	-0.79	-0.799	-0.817	-0.857
	P-value	0.12	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

PF, physical functioning; RP, role limitations due to physical health; RE, role limitations due to emotional problems; VT, vitality; MH, mental health; SF, social functioning; BP, bodily pain; GH, general health; FTMTRS, Fahn Tolosa Marin Tremor Rating Scale; NMSS, non-motor symptom scale; SD, standard deviation.

19 patients with *de novo* PD, 35 patients with advanced PD and 35 healthy controls in which the serum α -syn levels in healthy controls were greater than those in patients with ET and PD (12). Another recent study investigated erythrocytic total and aggregated α -syn and showed increased concentrations in patients with ET and PD compared with controls. Remarkably, compared with patients with PD, patients with ET showed significantly greater erythrocytic total α -syn and lower ratios of erythrocytic aggregated to total α -syn, with a significant correlation between aggregated α -syn and disease duration (18). These studies demonstrated the potential role of α -syn and its variants in differentiating between ET and PD.

The lower α -syn values could be explained by reported Lewy pathology in the brains of patients with ET, particularly in the brainstem. Recently, ~25% of ET patients were reported to have Lewy pathology, which is heterogeneous and associated

with conversion to PD (8). The findings of the present study support the possibility of a link between ET and PD (19). The increasing evidence of changes in α -syn levels in patients with ET is a confirmation of such discrepancies in the pathogenesis of both diseases (12,18). However, further studies are required to confirm the role of α -syn as a diagnostic biomarker of ET that might differentiate it from other diseases or as a biomarker for the conversion of ET to PD.

Interestingly, serum α -syn did not correlate with tremor severity or other clinical characteristics, indicating its minor role in the pathogenesis of the disease or disease progression. Some studies of serum α -syn in PD patients reported a moderate correlation with disease severity, indicating a different role of Lewy pathology in the two diseases (9).

Moreover, the present study revealed similar serum levels of anti- α -syn autoantibodies between patients with ET and

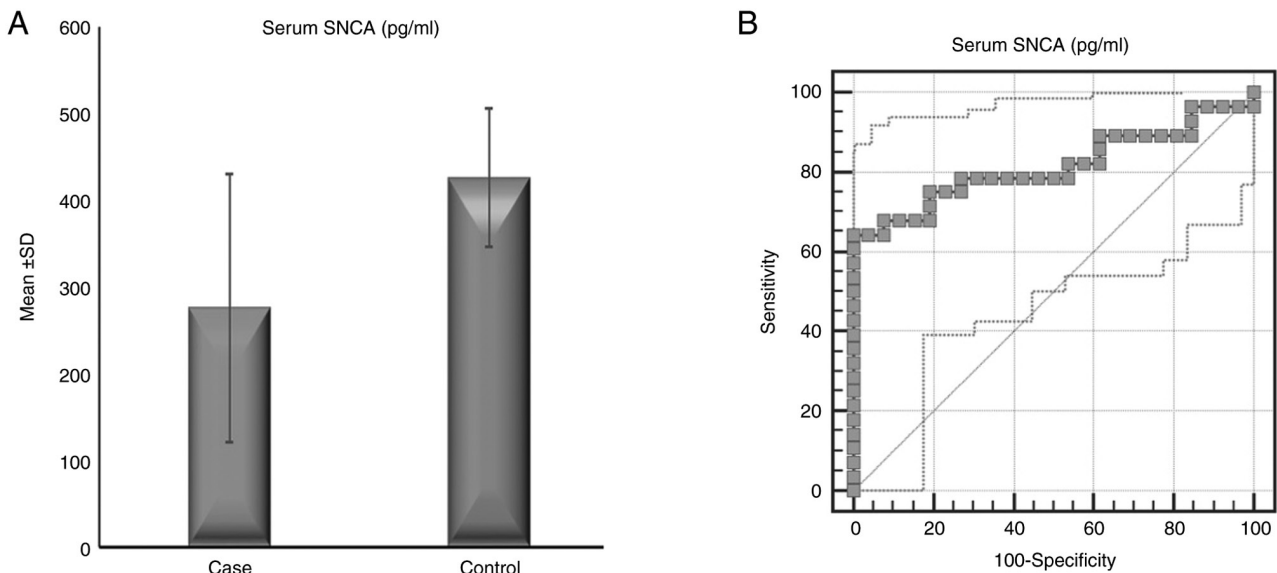


Figure 1. (A) Comparison of serum α -syn between patients with essential tremor and controls. (B) Sensitivity and specificity of serum α -syn. A-syn, α -synuclein.

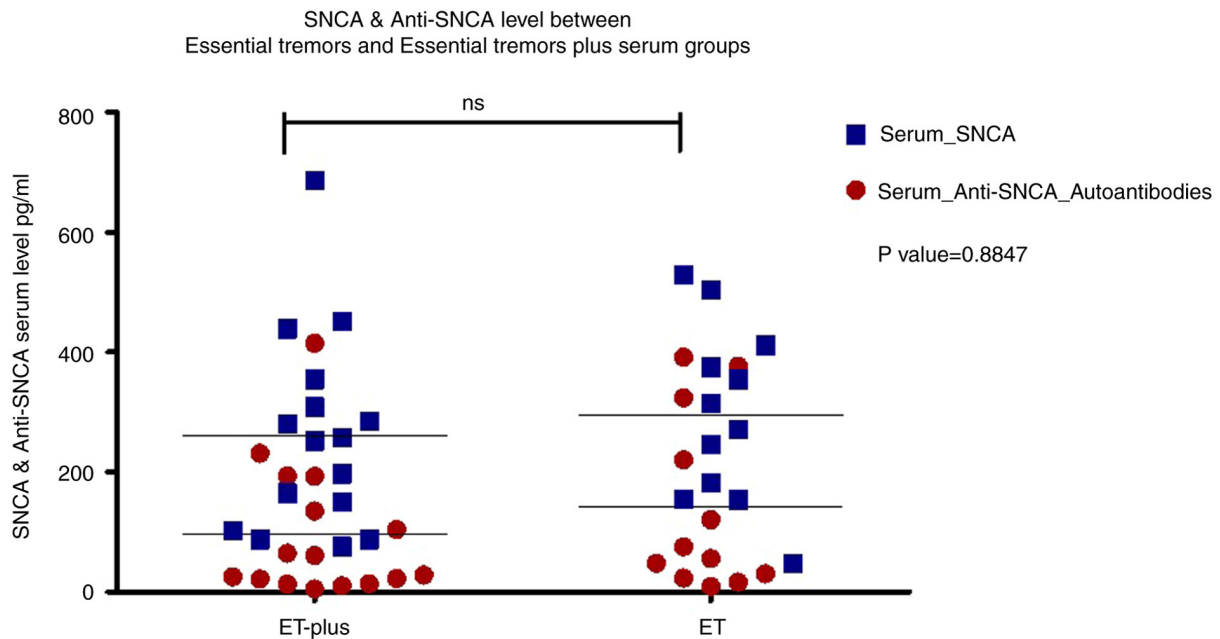


Figure 2. A comparison of serum levels of SNCA and anti-SNCA autoantibodies between the ET and ET-plus groups indicates that there is no statistically significant difference between the two groups ($P=0.8847$). SNCA, α -synuclein; ET, essential tremor.

controls. By contrast, patients with other neurodegenerative disorders, particularly PD, exhibit increased or decreased levels of anti- α -syn autoantibodies (9,10,20) suggesting the need for further studies to investigate the use of anti- α -syn as a differentiating biomarker between ET and PD. However, variable results of serum α -syn and its autoantibodies in PD patients have been reported, indicating the need for large studies with proper methodologies (9,20). To the best of the authors knowledge, no previous study has investigated serum anti- α -syn autoantibodies in patients with ET.

The present study confirmed worse NMSs, including cognitive impairment, depression and anxiety, and QoL, in patients with ET than in healthy controls, similar to the findings of

previous studies (4,21-24). Moreover, motor severity was related to different NMSS domains but not depression or anxiety, while QoL domains were related to different NMSs, cognitive impairment, depression and anxiety. On the other hand, tremor severity was correlated with only the vitality domain of QoL.

Previous studies have reported variable correlations between tremor severity and ET, owing to variability in clinical characteristics, variable assessment tools and small numbers of different cohorts. Similarly, in the present study, cognitive impairment and depression were not correlated with tremor severity, similar to the findings of previous studies (23-25) but were consistent with the findings of other studies (4,26).

Similarly, the relationship between anxiety and tremor severity was variable in previous studies (4,22). However, the findings of the present study are consistent with most studies showing that NMSs, including cognitive impairment, depression and anxiety, are the main determining factors of ET patients' QoL (4,21,22). The findings of the present study support that the associated NMSs of ET are inherent characteristics of the disease rather than secondary phenomena, which can be explained by dysfunction of the frontocerebellar circuits (4,25,27). The small sample size was the main limitation of the present study. In addition, adding a PD group is important for confirming the potential differentiating role of α -syn. Future larger studies are needed to confirm the role of the α -syn protein and its autoantibodies in ET.

In conclusion, the present study explored the serum levels of the α -syn protein and its autoantibodies in patients with ET and demonstrated that the serum α -syn level was lower in these patients than in controls, with no correlations with patient characteristics and similar anti- α -syn autoantibody levels. These findings suggest a potential role for serum α -syn as a biomarker for ET. Furthermore, profiling of proteins and their antibodies may provide a more holistic understanding of the disease process as well as the pathogenic involvement of the immune system. Additionally, the integral role of NMSs in ET and their negative impact on QoL was confirmed but no correlation with serum α -syn levels was observed.

The clinical implications of a study investigating the diagnostic value of serum alpha-synuclein (α -syn) to distinguish ET patients from healthy controls. This opens up several important possibilities: (i) Improved diagnosis: If α -syn levels are a reliable biomarker for ET, doctors could use them to diagnose ET sooner and more accurately. (ii) Improved treatment: Early diagnosis could allow doctors to start treatment sooner, which could improve outcomes for individuals with ET and Improved QoL: The present study also revealed that patients with ET had worse cognitive problems, emotional problems, and NMSs (such as fatigue and sleep problems), as well as a lower QoL. Knowing this could help doctors provide improved support and care for individuals with ET, improving their daily lives.

The present study is piloting the use of serum α -syn as a potential biomarker for ET. The results are still considered very early to reflect on clinical relevance, given absence of correlation to any of the clinical parameters measured. The present study was considered, as a trigger provider for future research to delve deeper into clinical and patho-mechanistic conclusions.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AS and EH conceptualized the present study. AS and AD organized the research project. AS, AD and MS executed the research project. AS and EH supervised and acquired the resources. AS, AD and MS designed the statistical analysis. AS and MS confirm the authenticity of all the raw data. AS and MS performed the statistical analysis. AS, AD, MB and EH reviewed and criticized the statistical analysis. AS and AD wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the Ain Shams University faculty of medicine (Cairo, Egypt) (approval no. FMASU MS 700/2021), and written informed consent was obtained from all patients. The authors confirm that they have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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