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Original article

Role of Tumor Necrosis Factor Alpha Induced Protein-8 Like-2 as a Biomarker of Parkinson's Disease

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Abstract

Background and Aim: Neuroinflammation plays an early and prominent role in the pathology of Parkinson disease. Tumor necrosis factor alpha induced protein-8 like-2 (TIPE2) is a relatively new subtype of tumor necrosis factor which may play a role in pathogenesis of Parkinson disease. Our aim was to evaluate the role of serum level of TIPE2 as a risk factor for Parkinson disease and as a serological biomarker of disease severity. **Methods:** Forty-seven patients diagnosed as idiopathic PD according to diagnostic criteria of the UK Parkinson Disease Society Brain Bank, and 47 healthy individuals were enrolled. All patients were on medical treatment of PD and were evaluated by Unified

Parkinson's disease Rating Scale (UPDRS), and Modified Hoehn and Yahr staging scale(HY). Cognitive function was assessed using Montreal Cognitive Assessment Scale (MOCA). TIPE2 serum level was measured in all participants. **Results:** PD patients had significantly higher levels of TIPE2 (P-value <0.001). Also, PD patients with cognitive impairment had significantly higher levels of TIPE2 (P-value= 0.039). TIPE2 level was positively correlated with score of modified HY staging (p-value o.oo1). Also,TIPE2 level was positively correlated with bradykinesia, total motor sub scores of UPDRS and total score of UPDRS (p-value 0.009, 0.019, 0.027 respectively). There was a significant negative correlation between TIPE2 and the scores of executives and visuospatial functions, attention, abstraction and total score of MoCA. **Conclusions:** TIPE2 serum levels in PD patients are higher than its serum level in healthy controls. Such high level of TIPE2 has a considerable impact on disease severity.

1. Introduction:

PD is a prevalent neurodegenerative disorder, presents with heterogeneous symptomatology that includes motor and non-motor symptoms. Its pathogenesis involves different molecular pathways, involving Lewy bodies (LBs) and early loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), and subsequent deficiency of dopamine in the basal ganglia (BG).[1]

The PD pathology starts 20 years earlier than motor manifestation. While nigrostriatal degeneration can be detected 5–10 years before symptomatology, using different imaging and pathology techniques of investigations.[2] In the late stage of PD, the impact of cognitive impairment is particularly relevant. The inflammatory biomarkers detected by imaging techniques or by sample analysis

are now considered valuable tools for PD diagnosis and prognosis.[3] TIPE2 has been identified as a negative regulator of innate and adaptive immunity. Its expression is by lymphoid and nervous tissues where it maintains hemostasis of immunity.[4] TIPE2 exerts its action through maintaining phagocyte bactericidal activity and regulating apoptosis and inflammatory cascade. TIPE2 which is produced by hematopoietic cells, controls inflammation by inhibiting T cell receptors as well as Toll-like receptors via nuclear factor-kappa-B and C signaling mechanisms of Jun N-terminal kinase.[5]

The aim of this work was to evaluate the role of serum level of Tumor Necrosis Factor Alpha induced protein-8 like-2 (TIPE2) as a risk factor for Parkinson disease and as a serological biomarker of disease severity

2. Patients and Methods:

A case-control study, conducted at neurology department, Beni-Suef University hospital during the period between April 2019 and September 2020. It was ethically approved by Faculty of Medicine, Beni-Suef University Research Ethical Committee (Approval number: FMBSUREC/05032019) from the ethical point of view.

The study was explained to all participants and informed consent was taken from them before starting study.

The total number of participants was 94; 47 egyptian patients diagnosed as PD according to clinical diagnostic criteria of the UK Parkinson Disease Society Brain Bank,[6] and all patients were on treatment of PD, and 47 healthy volunteers matched for age and sex from the same geographical area as a control.

Patients having these diseases, were excluded from this study: severe physical, hearing, or visual impairment that interferes with their ability to finish testing. Patients with secondary PD (Drug-induced, post traumatic, or post infectious) or atypical PD, Patients who have had a concurrent stroke, Patients with MRI brain showing abnormal lesions.

All Parkinson disease patients were subjected to full history taking, clinical examination including the following:

- Evaluation and staging of PD using:
  - Modified Hoehn and Yahr staging scale: to evaluate the overall stage of PD regarding clinical characteristics and functional impairment.[7]
  - Unified Parkinson's Disease Rating Scale (UPDRS) was used as a rating scale for assessment of PD patients enrolled in our study (on medications).[8]

Neuropsychological scales and cognitive evaluation using the Montreal Cognitive Assessment (MoCA**)** Arabic version. The MoCA is a 30-item exam that assesses seven cognitive domains: visuospatial/executive functioning, naming, memory, attention, language, abstraction, and orientation. It was created by Nasreddine and colleagues as a mild cognitive impairment (MCI) screening test. [9] This test is sensitive to MCI and can predict future cognitive decline in a variety of cognitively impaired states, including Alzheimer's disease, and its process time is 1o–15 minutes with a cut off value < 26.

Both patients and control groups were subjected to evaluation of Serum levels of Tumor necrosis factor-α-induced protein-8 like-2 (TIPE2) by enzyme-linked immune-sorbent assays (ELISA) using by commercially available ELISA kit (My Biosource, USA) regarding to manufacturer's guidelines and past studies.[10] The serum was separated then the samples were stored frozen at −80°C freezer until use for measuring TIPE2. The kit uses a double-antibody sandwich ELISA to assay the level of Tumor necrosis factor-α-induced protein-8 like-2 (TIPE2) level in samples.

- The plate has been pre-coated with human TIPE2 antibody. TIPE2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human TIPE2 antibody is added. The streptavidin-HRP is added and binds to biotinylated TIPE2 antibody. Unbound streptavidin-HRP is washed away. Substrate solution is added and color develops in proportion to the amount of human TIPE2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.
- The statistical analysis was done using SPSS (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago, IL, USA). The qualitative data were described in the form of Mean (SD) and range. independent t test, was used to assess the statistical differences between values. ANOVA test was used to compare stages of H &Y in TIPE2 level. Pearson correlation was used to correlate TIPE2 serum level with clinical scales. P value of less than 0.05 was considered as statistically significant.

3. Results:

A total 94 individuals enrolled in the current study, the age of PD patients

ranged from (45 to 72) years with a mean value of 60.48(SD=6.88) years, and the range of age of control group ranged from (41 to 71) years with a mean value of 59.61(SD=7.o3) years. Male: female ratio in PD and control groups was 2.9:1 and 2.6:1, respectively.

Regarding clinical assessment scales of PD patients, the score of modified HY staging scale for PD patients with a range of 1 to 5 and with a mean value 2.29(SD=1.24). The total score of UPDRS in PD patients ranged from 13 to 121 with a mean of 59.51(SD=28.96). The total score of MoCA ranged from 12 to 3o with a mean value 2o.31(SD= 6.o4) **(Table 1).**

Table 1: Clinical and Cognitive assessment scales of PD patients.

			PD patients (n=47)	
			Range (minimum-maximum)	**Mean (SD)**
Modified HY staging scale			(1-5)	2.29±1.24
UPDRS	**Motor score**	**Tremor**	(1-17)	5.o4±3.79
		Rigidity	(0-16)	6.95±4.53
		Postural instability	(0-4)	1.53±1.24
		Bradykinesia	(4-41)	18.48±9.71
		Total score motor	(7-71)	35.01±16.57
	Mentation score		(0-14)	4.72± 3.65
	Activities score		(2-39)	17.55±8.77
	Medication complication		(0-11)	2.61±2.57
	other complication		(0-3)	1.21±0.92
	Total-UPDRS		(13-121)	59.51±28.96
MoCA	**Executive and visuspatial functions**		(0-5)	3.02 ± 1.55
	Naming		(2-3)	2.46 ± 0.50
	Attention		(0-6)	4.0 ± 2.04

	Language	(0-3)	2.0 ± 0.97
	Abstraction	(0-2)	0.97 ± 0.76
	delayed recall	(0-5)	2.55 ±1.50
	orientation	(2-6)	5.29 ± 1.30
	Total MoCA score	(12-30)	20.31± 6.04

HY: Hoehn and Yahr, UPDRS:Unified Parkinson's Disease Rating Scale, MoCA: Montreal Cognitive Assessment PD: Parkinson's disease

The mean value of serum TIPE2 for PD patients were 73.24 mg/dl (SD=7.31) and ranged from 12.8 to 167.5 mg/dl while the mean value for controls were 36.35 mg/dl (SD=2.6) and ranged from 14.5 mg/dl to 89.6 mg/dl. The PD patients had a significant higher TIPE2 serum levels than controls (P-value <0.001) (Table 2).

Table 2:TIPE2 serum level mean value for PD patients and controls

TIPE2 (mg/dl)	**Patients (n=47)**	**Control (n=47)**	**P value**
Mean ±SD	73.24 ±7.31	36.35 ±2.6	<0.001**
Range (Min-Max)	154.7 (12.8-167.5)	75.1 (14.5-89.6)	

TIPE 2: Tumor Necrosis Factor Alpha Induced Protein-8

Patients with severe HY score had a statistically significant higher TIPE2 measurement [117.74 (SD=45.98)] mg/dl than patients with moderate HY score [91.42(SD=66.93)] mg/dl or mild HY score [57.09(SD=38.84)] mg/dl**.** Male patients had TIPE2 measurement of [71.26 (SD=51.89)] mg/dl and female patients [73.35(SD=42.87)] mg/dl, with no statistically significant difference. (Table 3)

Patients with cognitive impairment had a statistically significant higher TIPE2 serum level measurement [84.1 mg/dl (SD=9.41)] than patients without cognitive impairment [52.4 mg/dl (SD=9.68)] (P value 0.039), with cutoff point: MoCA score 26 **(Table 3)**.

Table 3: Serum levels of TIPE2 according to different variables in patients with PD.

Variables		Serum levels of TIPE2 (Sum {Mean±SD })	P value
HY	Mild (n=33)	57.09±38.84	0.003*
	Moderate (n=6)	91.42±66.93	
	Severe (n=8)	117.74±45.98	
Sex	Male (n= 35)	71.26±51.89	0.9
	Female (n= 12)	73.35±42.87	
Cognitive impairment	Yes	84.1 ±9.41	0.039*
	No	52.4 ±9.68	

HY: Hoehn and Yahr

TIPE2 level was significantly positively correlated with score of modified HY staging (p-value 0.001). Also, TIPE2 was significantly positively correlated with bradykinesia and total motor sub scores of UPDRS and total score of UPDRS (p-value 0.009, 0.019, 0.027 respectively). There was a significant negative correlation between TIPE2 and the scores of executives and visuospatial functions, Attention, Abstraction and Total score of MoCA **(Table 4).**

Table 4: Correlation of TIPE 2 serum level and clinical assessment scales in PD patients.

Motor assessment	TIPE 2	
	(r) coefficient	P- value
Modified HY staging scale	0.408*	0.001*

UPDRS	**Motor**	**Tremor**	0.451	0.25
		Rigidity	0.17	0.465
		Postural instability	0.109	0.053
		Bradykinesia	0.378*	0.009*
		Total score	0.34*	0.019*
	Mentation		0.279	0.057
	Activities		0.287	0.051
	Complication of medication		0.121	0.42
	other Complication		-0.137	0.359
	Total UPDRS		0.323*	0.027*
MoCA score	**executive and visuspatial functions**		-0.366*	0.011*
	Naming		-0.267	007
	Attention		-0.361*	0.013*
	Language		-0.21	0.157
	Abstraction		-0.315*	0.031*
	delayed recall		-0.197	0.185
	orientation		0.197	0.184
	Total MoCA score		-0.319*	0.029*

(r) using Pearson coefficient p-value ≥ o.o5 (non-significant)

HY: Hoehn and Yahr UPDRS: Unified Parkinson's Disease Rating Scale; TIPE2: tumor necrosis factor alpha induced protein 8 like 2, MoCA: Montreal Cognitive Assessment, TIPE2: tumor necrosis factor alpha induced protein 8 like 2

4. Discussion:

TIPE2 is one of the cytokine family tumor necrosis factor-induced protein-8 (TNFAIP8) where it maintains immunological hemostasis by inhibiting the Phosphoinositide 3-kinase–Rac signaling pathway.[11] TIPE2 suppresses the activation of NF-B at the molecular level, resulting in anti-inflammatory actions, reduced apoptosis, and dopaminergic neuronal death.[12]

Studies which evaluated the potential role of TIPE2 in pathogenesis and disease severity in Parkinson disease are few, to our knowledge and after meticulous review of literature, this study is the 2nd study to discuss this topic after the study of Kouchaki and colleagues

In our study, PD patients were found to have a significantly higher TIPE2 serum level than age matched controls (p=0.001*).

Against our findings, the study conducted by Kouchaki and colleagues who concluded those changes in mean TIPE2 serum levels between Parkinson's disease patients and healthy controls were non-significant. Furthermore, they discovered no significant variations in TIPE2 circulatory gene expression between patients and controls.[10]

Some explanations for this discrepancy may include: racial differences, which may affect the genetic expression of TIPE2.

Considering such effects, TIPE2 as a prominent inhibitor of T-cell receptor and Toll-like receptor signaling. Toll like receptors have roles in inducing inflammation and oxidative stress in different neurological diseases. Also, TIPE2 promotes of angiogenesis, which is considered in PD pathogenesis as new blood vessels formation leads to more tissue repair.[10] Moreover, longitudinal assessment and further follow up of the serum level may elucidate its role.

In our study, patients with cognitive impairment were found to have a statistically significant higher TIPE2 than patients without cognitive impairment

Additionally, in the present study, TIPE2 was significantly negatively correlated with the scores of executives and visuospatial functions, Attention, abstraction and Total score of MoCA.

Moreover**,** we found no statistical difference regarding both serum level of TIPE2 and cognitive performance between male PD patients and female patients.

Previous research on cognitive decline to dementia had found gender disparities in cognitive performance in PD. Cereda and colleagues discovered that male gender

was a risk factor for dementia in Parkinson's disease.[13]

Similarly, Cholerton and colleagues discovered that male gender was a predictor of cognitive deterioration from non-impairment to MCI, as well as MCI to PDD.[14] Additionally, males showed a faster trend of cognitive deterioration in terms of processing speed and working memory.

However, Gao and colleagues found that cognitive problems were more serious in Chinese females with Parkinson's disease than in males. Females exhibited considerably lower MoCA scores after adjusting for illness period and years of education.[15]

In contrast to our findings, Gennatas and colleagues discovered that women may experience quicker cognitive decline than males due to variations in sex hormones, structural development of the brain, genetic profile, psychosocial status, lifestyle factors, functional connectivity, and tau pathology.[16] Such differences are multifactorial, which need more adjustment, bigger sample size and more correlation studies.

Our study revealed that patients with severe H&Y score were found to have a statistically significant higher TIPE2 serum level than patients with moderate or mild H&Y score.

Zhang and colleagues considered that TIPE2 was thought to be a protective factor that inhibits the inflammatory state of PD. However, additional variables like TIPE2 genetic polymorphism and comorbidities may impact its serum level and explain this discrepancy.[17] Duration of the treatment, mainly due to the anti-inflammatory role of some PD medications, may influence the results, so comparative studies including on and off treatment patients are needed.

There was a statistically significant positive correlation between TIPE2 and bradykinesia and total motor sub scores of UPDRS and total score of, this may refer to the impact of TIPE2 serum level on motor symptoms.

The main limitation in this study is that we did not monitor the changes of TIPE2 longitudinally.Also, different molecular mechanisms, related to TIPE2 gene in PD should be investigated.—Consideration of comorbidities, different medications, and duration of medication are highly recommended in future studies.

5. Conclusions:

TIPE2 serum levels in PD patients are higher than its serum level in healthy controls. Such high level of TIPE2 has a considerable impact on both motor and cognitive symptoms. TIPE2 molecular

pathways should be further evaluated in PD.

List of Abbreviations:

BG; Basal ganglia

ELISA; Enzyme-linked immune-sorbent assays

LBs; Lewy bodies

MCI; Mild cognitive impairment

MOCA; Montreal Cognitive Assessment Scale

PD; Parkinson's disease

SNpc; Substantia nigra pars compacta

TIPE 2; Tumor Necrosis Factor Alpha Induced Protein-8

TNFAIP8; Tumour necrosis factor-induced protein-8

UPDRS; Unified Parkinson's disease Rating Scale

HY score; Hoehn and Yahr

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