Original Article

Pro-inflammatory cytokines levels in tears and dry eye disease in Parkinson's disease

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Background: Neuroinflammation is an essential event in Parkinson's disease (PD). Identifying affordable and less invasive biomarkers to make an early diagnosis and monitor therapeutic strategies should be a priority among researchers. The study's objective was to measure tear levels of cytokines in subjects with PD and their association with motor features and the presence of dry eye symptoms.

Methods: A total of 16 subjects with PD and 16 age- and sex-matched controls were included. Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), Hoehn and Yahr (HY) stage scale, Montreal Cognitive Assessment (MoCA), tear break-up time (TBUT), blink rate (BR), Dry Eye Questionnaire 5 (DEQ-5) were examined, and pro-inflammatory cytokines [interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70 and tumor necrosis factor-alpha (TNF-α)] were quantified in tears using the BD Cytometric Bead Array Human Inflammatory Cytokine Kit.

Results: Higher tear TNF-α were quantified in PD compared to controls (2.94±3.95 *vs.* 0.33±0.49 pg/mL, P=0.008). According to DEQ-5, 50.0% (n=8) of PD subjects and 12.5% (n=2) controls had dry eye disease (DED). No differences were found in cytokines concentrations between PD patients with DED compared to those without DED. IL-8 was associated with the HY stage, TBUT, DEQ-5, and a better MoCA score. A higher BR correlated moderately with a lower HY stage (r=–0.645, P=0.007), and DED patients have lower BR in PD (12.14±2.54 *vs.* 9.0±2.06 blinks/minute, P=0.031).

Conclusions: PD patients have higher levels of TNF-α in tears than age- and sex-matched HC. IL-8 in tears may be both involved in the severity of the disease and in the development of DED in PD. In addition, our findings suggest that as HY stage increases, indicating a more advanced stage, BR decreases, indicating greater motor impairment. Conversely, the presence of DED is associated with higher levels of bradykinesia

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in PD patients, suggesting a potential relationship between DED and motor impairment severity.

Keywords: Tears; pro-inflammatory cytokines; dry eye disease (DED); Parkinson's disease (PD)

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Introduction

Parkinson's disease (PD) affects around 1% of individuals above 60 years of age; it is the second most common degenerative disorder (1). Bradykinesia, tremor, and rigidity are the primary motor symptoms in these patients. The prodromal phase is characterized by non-motor symptoms such as olfactory dysfunction, psychiatric symptoms, autonomic system dysfunction, sleep disorders, pain, and fatigue (2). The pathogenesis of PD has not been clarified in detail. Evidence supports the link of PD with chronic neuroinflammation and microglia activation. Increased levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12p70, and interferon-gamma (IFN-γ) can stimulate the recruitment of peripheral leukocytes to the central nervous system. A permanent pro-inflammatory state results in chronic inflammation (3-5).

Moreover, high levels of specific cytokines have been linked to the severity of motor and non-motor symptoms such as depression, fatigue, and cognitive impairment in PD (6-10). Increased levels of cytokines IL-1β, IL-2, IL-4, and IL-6 in cerebrospinal fluid (11,12) and in the striatum of the postmortem brain of PD patients have been reported (13).

PD patients have been reported to present a higher prevalence of ophthalmologic conditions such as dry eye disease (DED) than control patients (60–86% *vs.* 5–30%) (14,15). DED in PD has been explained by a decreased blink rate (BR) and subsequent reduction of the lipid layer over the cornea, which leads to the aqueous layer evaporating. Moreover, reduced tear production due to autonomic dysfunction has been reported (16,17). Pathogenesis of DED is associated with ocular surface inflammation. Several pro-inflammatory cytokines have been found elevated in tears from DED subjects (18).

TNF-α is considered a major pro-inflammatory cytokine in neuroinflammation. TNF-α and other cytokines are released for neuroprotection, but in later stages, they become neurotoxic (19). In addition, in one study of postmortem PD brains, the areas with significant loss of dopaminergic neurons were those with the highest levels of TNF-α (20-22). Interestingly, elevated levels of TNF-α have been found in the tears of PD patients compared with healthy controls (HC) (23). These increased levels of cytokines in the tears of PD patients may be explained by the underlying pathophysiology, bradykinesia, and DED. The current study was designed to quantified the levels of cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNF-α) in tears in PD and HC, and to evaluate whether these inflammatory cytokines correlated with disease clinical characteristics and DED indicators. We hypothesized that PD patients will have higher levels of cytokines in tears in comparison to HC and that these levels will correlate with motor severity and DED markers. To the best of our knowledge, this is the first study to investigate the quantification of the pro-inflammatory profile in tears and also the existence of DED in PD patients. DED is usually linked to an inflammatory profile and is seen frequently in patients with PD. We present this article in accordance with the STROBE reporting checklist (available at https://aes.

Highlight box

Key findings

- Parkinson's disease (PD) patients have higher tumor necrosis factor-alpha (TNF-α) levels in their tears when compared to controls.

What is known and what is new?

- Neuroinflammation plays a critical role in PD, and pro-inflammatory cytokines are key players in the immune response.
- Patients with PD exhibit elevated levels of TNF-α in tears, suggesting the potential involvement of this cytokine in neuroinflammation in PD.

What is the implication, and what should change now?

- The elevated TNF-α levels in tears of PD patients suggest that it could serve as a potential biomarker for neuroinflammation in PD.
- Monitoring tear cytokine levels could aid in early diagnosis and monitoring therapeutic strategies for PD.

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Methods

Subjects and controls

Sixteen subjects with idiopathic PD and 16 age- and sex-matched HC were included. They attended the Movement Disorder Outpatient Clinic at the National Institute of Neurology and Neurosurgery in Mexico City and were enrolled. HC were volunteers recruited from an ophthalmological hospital. Written consent was obtained from all participants. This study was performed following the ethical standards of the Helsinki declaration (as revised in 2013) and was approved by the institutional review board and local ethics committee of the National Institute of Neurology and Neurosurgery, Mexico City, Mexico (No. 162-19).

All subjects underwent medical history evaluation and physical, neurological, and ophthalmological examinations. Patients were assessed according to the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnosis Criteria to confirm the idiopathic PD diagnosis. We included demographic information like age, age at disease onset, PD duration, and predominant side of symptoms. We recorded Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (24), this is a comprehensive assessment tool designed to evaluate various motor and non-motor aspects of PD. It consists of four parts that assess different domains of the disease. Part I—non-motor experiences of daily living: this part assesses the impact of non-motor symptoms on daily activities and quality of life. It includes questions related to mood, cognition, hallucinations, apathy, sleep disturbances, and other non-motor aspects. The scale evaluates the frequency and severity of these symptoms and their impact on the patient's functioning. Part II—motor experiences of daily living: this section focuses on motor symptoms and their impact on activities of daily living. It covers areas such as speech, salivation, swallowing, handwriting, cutting food, dressing, hygiene, falling, and freezing of gait. The scale rates the severity and frequency of these motor symptoms and their impact on the patient's ability to perform daily tasks. Part III—motor examination: part III is a comprehensive assessment of motor symptoms using a standardized examination protocol. It evaluates various aspects of motor function, including tremor, rigidity, bradykinesia (slowness of movement), and postural instability. The examination assesses motor symptoms at rest, during posture maintenance, and during various movements. It provides a detailed evaluation of motor impairment and helps track disease progression. Part IV—motor complications: this part focuses on motor complications that may arise as a result of long-term treatment with dopaminergic medications. It assesses motor fluctuations (changes between "on" and "off" states) and dyskinesias (abnormal involuntary movements). The scale evaluates the frequency, severity, and impact of these complications on daily activities and quality of life. Each part of the MDS-UPDRS is scored independently, and the scores from all four parts are often combined to obtain an overall assessment of disease severity and progression. The scale provides a standardized and comprehensive evaluation of both motor and non-motor symptoms, helping clinicians and researchers assess the impact of PD and track changes over time.

Montreal Cognitive Assessment (MoCA) (25), and disease stage were evaluated by the Hoehn and Yahr (HY) stage scale (26). Scales were assessed by a movement disorder specialist. The permission for the use of scales was requested and obtained according to the instructions for members of the International Parkinson Movement Disorders Society. Postural instability and gait disturbance (PIGD) motor subtype, hallmarked by bradykinesia and axial symptoms, and PIGD score were determined according to Stebbins *et al.* (27).

Exclusion criteria for controls were subjects with familial history of PD, parkinsonism, tremor, dementia by Lewy bodies, Alzheimer's disease and/or any other alpha synucleinopathy, systemic diseases including inflammatory, autoimmune, hematologic, neoplasia disease, diabetes mellitus, thyroid disease, alcohol addiction, acute or chronic infection, hepatic of renal failure, use of diuretic, anti-inflammatory, antineoplastic, corticosteroid, immunosuppressive, antidepressant or anxiolytic drugs within the last 2 months; and use of topical drops within the last month. The control group was excluded if the systemic or ocular medication caused possible eye problems.

In addition, subjects with an active ocular infection or signs of inflammation, ocular allergy, pterygium, use of ophthalmic drops, use of contact lenses, or ocular surgery in the last 3 months were also excluded. Only the right eyes of PD and HC subjects were used for analysis.

Dry eye evaluation included the following

Tear break-up time (TBUT) and fluorescein staining

A sterile Ful-Glo® Fluorescein Sodium Ophthalmic Strip

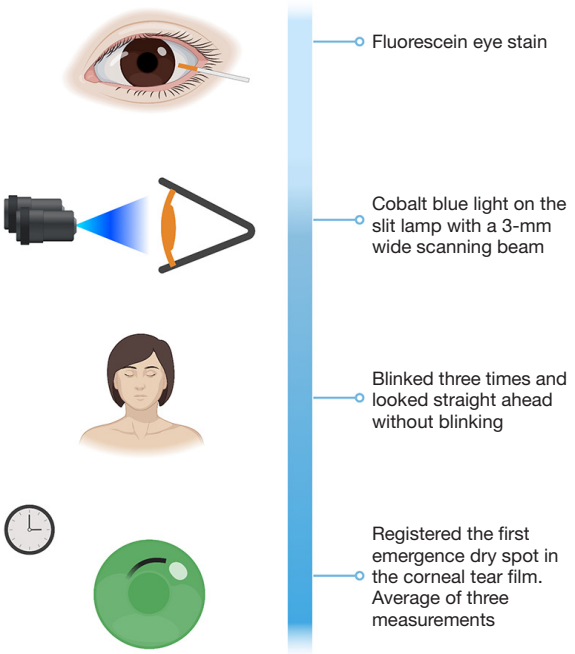

Figure 1 Tear film break-up time and fluorescein staining procedure.

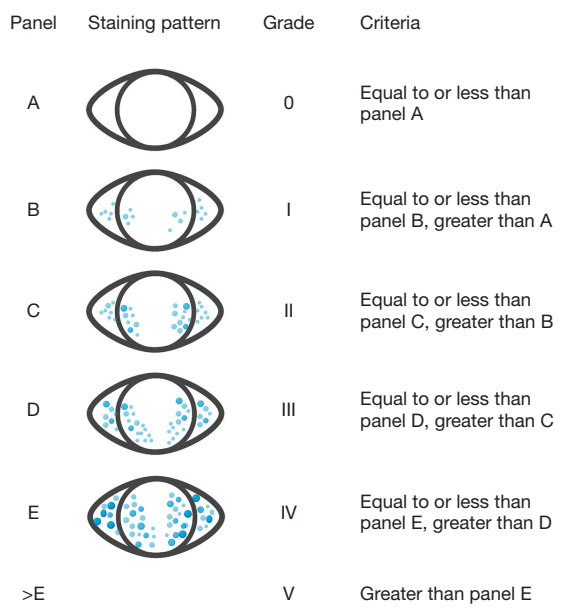

Figure 2 Grading of corneal and conjunctival staining (Oxford scheme).

(Sola/Barnes-Hind, Phoenix, AZ, USA) wetted with a drop of non-preserved buffered saline was placed into the external third of the lower eyelid. The TBUT measurement was then taken using the cobalt blue illumination on the slit lamp with a 3-mm-wide scanning beam in each eye. After the instillation of fluorescein, the participant was asked to blink three times and, after that, to look straight ahead without blinking. The time interval between a complete blink and the first emergence of a dry spot in the corneal tear film was measured. The average of three successive measurements of the TBUT test was calculated (*Figure 1*). The result was considered positive if TBUT was less than 10 seconds (28). Corneal staining was graded using the Oxford scheme (*Figure 2*). An increased staining panel labeled A to E according to the severity is used (A, less severe, E, more severe). Staining ranges from 0 to V for each panel and 0 to 15 for the entire exposed inter-palpebral area. The staining grade is represented by punctate dots, increasing by 0.5 of the logarithms of the number of dots between panels B and E (29).

BR

In the absence of a provoking external stimulus, a bilateral paroxysmal closure of the eyelids lasting less than one second was considered to be a blink. In a 3-minute video recorded while the patient was having a conversation, the "BR" was defined as the average rate at which the eyelids were closed per minute. Blinks were considered complete if at least half of them involved rapid eye reopening and eye closure (30,31).

Dry Eye Questionnaire 5 (DEQ-5)

The DEQ-5 (32) focuses on three common DED symptoms: eye discomfort, eye dryness, and watery eyes. Scores of ≥6 indicate mild dry eye symptoms, and scores of ≥12 indicate severe symptoms. For study purposes, DED was defined as positive symptoms measured by DEQ-5 and a decreased TBUT (<10 seconds) or ocular surface staining (Oxford schema ≥ grade III) (33).

Tear collection sample and flow cytometry analysis

Unstimulated 100 µL tear samples were collected non-traumatically from the outer canthus of open eyes using capillary tubes during the collection sample. Tear samples were stored at –80 ℃ until assayed (*Figure 3*). BD Cytometric Bead Array Human Inflammatory Cytokine Kit (BD Biosciences, USA) was used to assess the cytokine profile (allowing one to measure the concentration of IL-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α) in tear samples.

The tests were performed according to the manufacturer's protocols (in this link you can find the information on the cytokine kit: https://www.bdbiosciences.com/en-us/

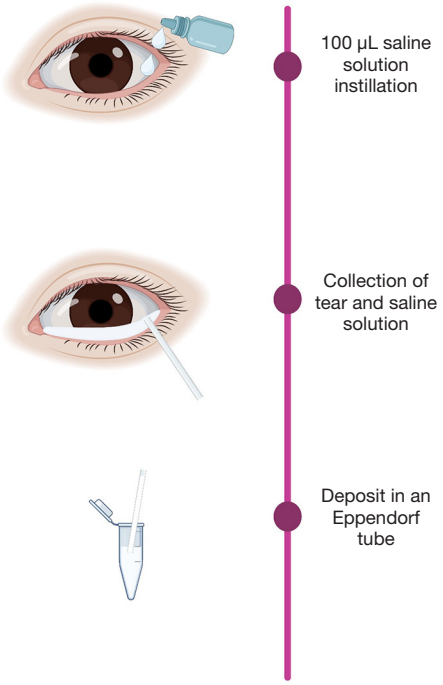

Figure 3 Tear collection sample.

products/reagents/immunoassay-reagents/cba/cba-kits/human-inflammatory-cytokine-cytometric-bead-array-cba-i-kit.551811). BD Cytometric Bead Array Human Inflammatory Cytokines Kit: 50 µL of assay beads and 50 µL of the studied sample was added consecutively to each sample tube. The samples were incubated at room temperature in the dark for 1.5 hours. Next, the samples were washed with 1 mL of Wash Buffer, centrifuged, and the resulting pellet was resuspended in 50 µL of detection reagent. The samples were further incubated for 1.5 hours, washed, and centrifuged. After discarding the supernatant, the pellet was resuspended in 300 µL of wash buffer and analyzed on the same day in a flow cytometer. Before the analysis, the cytometer was calibrated using set-up beads according to the manufacturer's protocol.

Statistical analysis

All statistical data analyses were performed with SPSS 25.0 Statistics Inc. and graphs were created with GraphPad Prism 9. The Shapiro-Wilk test was used to examine if the continuous variables were normally distributed. Demographic and clinical data were calculated with mean values and standard deviation for continuous variables. Wilcoxon matched pair signed rank (two samples) was used to compare cytokine concentrations between PD subjects and controls. Mann-Whitney *U* test was done to compare PD clinical characteristics between DED and subjects without DED. Correlation between cytokines concentrations and age, age on onset, PD duration, MDS-UPDRS I–IV, TBUT, BR, and DEQ-5 was assessed using Spearman's rank correlation coefficient. Statistical differences were considered significant if $P<0.05$.

Results

A total of 16 subjects with PD and 16 age- and gender-matched HC were included. The mean age of all PD subjects was 63.25±12.73 years. Regarding gender, 68.8% (n=11) were male and 31.3% (n=5) were female. The side of the initial onset of the disease was right at 56.3% (n=9). The demographic and clinical characteristics of PD subjects and HC divided by a group with DED and without are shown in *Table 1*.

Frequency of DED

According to DEQ-5, 50.0% (n=8) of PD subjects and 12.5% (n=2) controls had DED symptoms. In PD group, mild symptoms were present in 37.5% (n=6) and severe symptoms in 12.5% (n=2). The pro-inflammatory cytokine profile of HC with DED was: IL-1 (0.11±0.16 pg/mL), IL-6 (2.10±2.14 pg/mL), IL-8 (92.79±27.45 pg/mL), IL-10 (0 pg/mL), IL-12p70 (0.41±0.57 pg/mL), and TNF-α (0.1±0.01 pg/mL).

Statistical differences were found in TBUT, BR, and DEQ-5 when comparing PD subjects with PD. The mean of TBUT was lower in PD when compared to HC (8.18±4.03 *vs.* 13.5±3.74 seconds, P=0.001). TBUT was less than 10 seconds in 62.5% (n=10) of PD subjects and 12.5% (n=2) of controls. The mean DEQ-5 score was higher in PD than in HC (5.68±4.39 *vs.* 2.18±1.64, P=0.002). The mean BR was 10.37±2.68 blinks/minute in PD and 16.31±4.45 blinks/minute in HC (P=0.001).

The corneal fluorescein staining by Oxford schema the 25.0% (n=4) had a grade 0, 31.3% (n=5) had a grade I, 6.3% (n=1) had a grade II, 25.0% (n=4) had a grade III, and 12.5% (n=2) had a grade IV in PD group. In contrast to the control group, 43.8% (n=7) had a grade 0, 37.5% (n=6) had a grade I, 12.5% (n=2) had a grade II, and 6.3% (n=1) had a grade III.

A higher BR correlated moderately with a lower HY stage (r=–0.645, P=0.007).

Table 1 Demographic and clinical characteristics of PD and HC subjects with and without DED

Clinical characteristics	PD subjects with DED (n=8)	PD subjects without DED (n=8)	HC with DED (n=2)	HC without DED (n=14)
Sex, male (%)	66.7	71.4	50.0	71.4
Age (years)	64.25±14.08	62.25±12.10	80±9.89	60.85±11.40
Age of PD onset (years)	52.28±11.25	54.37±14.09	NA	NA
Duration of disease (years)	14.57±12.51	7.87±3.18	NA	NA
MDS-UPDRS I score	13.25±7.68	10.25±3.84	NA	NA
MDS-UPDRS II score	16.87±13.33	7.0±3.07	NA	NA
MDS-UPDRS III score	41.12±20.0	19.62±13.99	NA	NA
MDS-UPDRS IV score	4.0±4.98	0	NA	NA
MDS-UPDRS total score	62±35.79	26.62±16.48	NA	NA
HY stage	2.62±1.30	1.5±0.53	NA	NA

Data are presented as mean ± SD. PD, Parkinson's disease; HC, healthy control; DED, dry eye disease; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; HY, Hoehn and Yahr; NA, not applicable.

Eight PD patients and two controls fulfilled the study's operational definition of DED.

Comparison of cytokine profile between PD subjects and controls

The TNF-α concentration in tears was higher in PD subjects than controls (2.94±3.95 *vs.* 0.33±0.49 pg/mL, P=0.008). There was no difference between IL-1β, IL-6, IL-8, IL-10, and IL-12p70 concentration in tears of PD subjects *vs.* controls. These results are shown in *Figure 4*.

There was no evidence for a relationship between IL-1β, IL-10, and TNF-α tear concentration and any of the clinical characteristics assessed in PD subjects. In contrast, IL-6 positively correlated with duration symptoms (r=0.575, P=0.025). While tear concentration of IL-8 was correlated with age (r=0.545, P=0.029), HY (r=0.532, P=0.034), MoCA (r=–0.511, P=0.043), TBUT (r=–0.547, P=0.028), and DEQ-5 (r=0.605, P=0.013).

Comparison between PD subjects with and without DED

PD subjects with DED had a slower BR than PD without DED (8.87±2.16 *vs.* 11.87±2.47 blinks/minute, P=0.038). Regarding motor evaluation, PD subjects had the worst MDS-UPDRS III score (41.12±20.0 *vs.* 19.62±13.99, P=0.007). Nevertheless, there was no difference in the PIGD score between groups (1.75±1.13 *vs.*1.31±1.90, P=0.195) nor in the frequency of PIGD subtype (P=0.315). No differences were found in cytokine concentrations in tears between PD patients with DED compared to those without DED. These comparisons are presented in *Table 2* and *Figure 5*.

Discussion

Biomarkers are surrogate indicators of both standard and pathogenic biological processes. They are helpful for an early and accurate diagnosis, predicting the severity of the disease, and identifying effective therapeutic interventions (34). In PD identifying new biomarkers has become increasingly helpful over the past decades. These can help to detect patients at risk and may allow for an early diagnosis and management (35). The lacrimal tear is a biological fluid that can easily be collected and is considered a more accessible, less invasive, and less complex biological fluid than other tissues (36,37).

Cytokines are immunological messengers that contribute to neuroinflammatory cascades in PD. Several studies showed increased cytokines levels in PD subjects compared with controls in serum, cerebrospinal fluid and tears. Pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α are produced by microglia in the substantia nigra (SN); also, anti-inflammatory cytokine-like IL-10 is present in PD subjects (38-40). It is reported that these cytokines are linked to microglial activation as a consequence of excessive localized brain inflammation and neurotoxicity in neurodegenerative diseases (41).

IL-1β is a pro-inflammatory cytokine implicated

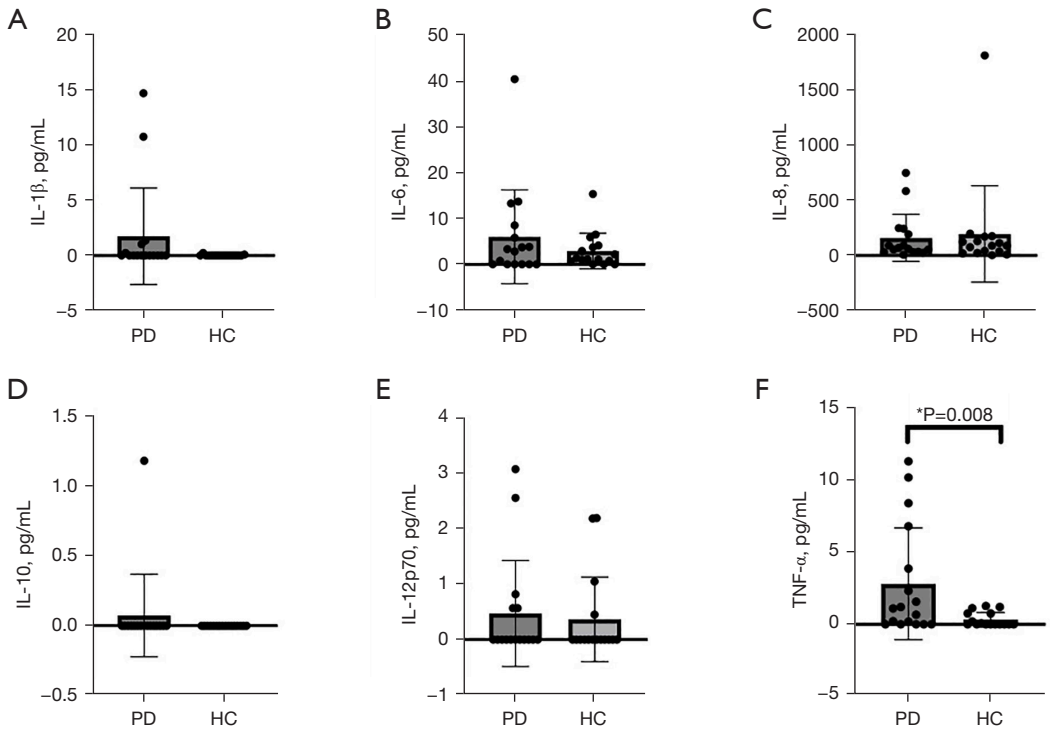

Figure 4 Comparison of cytokine profile concentration (y-axis) between PD subjects and controls (x-axis). n=16; *, P<0.05. IL, interleukin; PD, Parkinson's disease; HC, healthy controls; TNF-α, tumor necrosis factor-alpha.

Table 2 Comparison between PD subjects with and without DED

Clinical characteristics and cytokines	DED (n=8)	Non-DED (n=8)	P value
Age (years)	64.25±14.08	62.25±12.10	0.721
Years of PD	14.57±12.51	7.87±3.18	0.463
MDS-UPDRS I score	13.25±7.68	10.25±3.84	0.442
MDS-UPDRS II score	16.87±13.33	7.0±3.07	0.015*
MDS-UPDRS III score	41.12±20.0	19.62±13.99	0.007*
MDS-UPDRS IV score	4.0±4.98	0	NA
MDS-UPDRS total score	62±35.79	26.62±16.48	0.005*
HY stage	2.62±1.30	1.5±0.53	0.065
PIGD score	1.75±1.13	1.31±1.90	0.195
IL-1β (pg/mL)	1.37±3.78	2.13±5.10	0.645
IL-6 (pg/mL)	7.88±13.49	4.14±5.93	0.442
IL-8 (pg/mL)	209.23±232.65	107.16±194.06	0.065
IL-10 (pg/mL)	0	0.14±0.41	NA
IL-12p70 (pg/mL)	0.42±0.90	0.52±1.05	0.798
TNF-α (pg/mL)	1.28±1.32	4.61±5.04	0.645
Blink rate (blinks/minute)	8.87±2.16	11.87±2.47	0.038*

Data are presented as mean ± SD. *, P was considered significant when <0.05. PD, Parkinson's disease; DED, dry eye disease; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; HY, Hoehn and Yahr; IL, interleukin; PIGD, Postural Instability and Gait Disturbance; TNF-α, tumor necrosis factor-alpha; NA, not applicable.

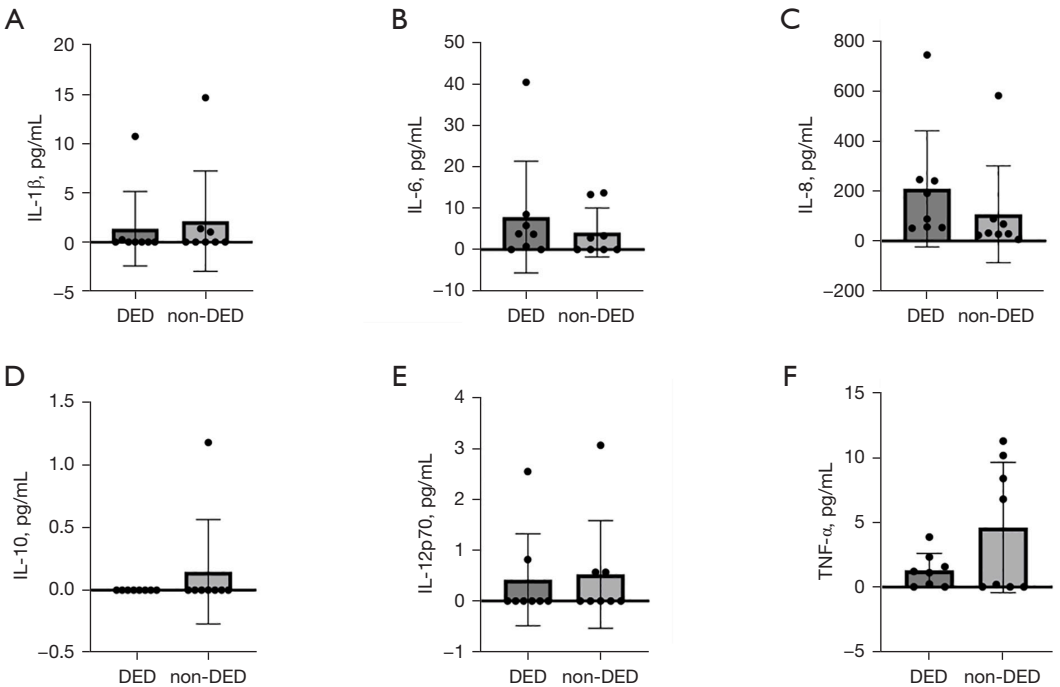

Figure 5 Comparison of cytokine profile (y-axis) between PD subjects with and without DED (x-axis). n=8. IL, interleukin; DED, dry eye disease; non-DED, without dry eye disease; TNF-α, tumor necrosis factor-alpha; PD, Parkinson's disease.

as the main effector of the functional consequences of neuroinflammation on neurodegeneration in PD models. Prolonged pro-inflammatory IL-1β expression in the SN results in significant and permanent dopaminergic neuronal death in the SN (42).

IL-8 is a chemokine produced by macrophages. Its receptor, the CXCR2 has been detected in dystrophic neurites, suggesting that IL-8 mediates glial interactions with neurons and thereby contributes to neuronal damage (43). Previous studies have shown inconsistent results of serum IL-8 levels in PD. Williams-Gray *et al.* (44) and Fu *et al.* (45) reported no statistical difference, but Gupta *et al.* (46) showed that IL-8 was significantly decreased in the serum in PD compared to controls (patients =222.70±200.91 pg/mL, controls =1,584.44±504.44 pg/mL; P<0.001). Also, serum IL-8 level was positively associated with disease duration, depression, and UPDRS III in PD patients (46,47).

IL-10 is an anti-inflammatory cytokine modulator of glial activation in neurodegenerative diseases. High levels have been reported in PD compared with HC (48). However, in another study, it was described that PD patients with more severe clinical characteristics and a non-tremor type showed reduced serum IL-10 levels (49).

TNF-α is a pro-inflammatory cytokine strongly correlated with PD pathology. In pathological conditions, astrocytes and microglia release large amounts of TNF-α. This process could contribute to the neuroinflammation seen in PD (21,50).

On the other hand, PD patients can concomitantly present DED, which is accompanied by a similar cytokines profile. The study's objective was first to determine the levels of cytokines in tears and compare them with age- and sex-matched controls and second, to correlate those findings with the motor symptoms and DED in PD. In our study, analogous to the literature, TNF-α tear levels were notably higher in PD subjects than in controls. Nevertheless, these levels were not linked to age, gender, age at onset, PD duration, motor symptoms, cognitive symptoms, or HY stages (23).

As expected, DED was more frequent in PD subjects than in control subjects, similar to what was reported in the literature, estimated at 60–86% in PD (14). Also, PD subjects had poorer TBUT and higher DEQ-5 scores than controls. Söğütlü Sarı *et al.* (30) found decreased levels of TBUT in 37 PD subjects than in controls (11.3 *vs.* 12.8 seconds), but the difference was not significant. Similarly, Reddy *et al.* (16) described no significant difference in TBUT (8.4±1.9 *vs.* 9.8±0.4 seconds) in PD

patients. However, Biousse *et al.* (51) reported that BR and TBUT were decreased in 30 PD patients compared with controls in untreated early-onset PD subjects.

In PD incomplete blinking and a decrease in BR can be observed due to a deficiency in dopamine. These changes could lead to tear film hyperosmolality, accelerated tear evaporation, and finally corneal damage causing DED (52). In our study, a higher BR correlated moderately with a lower HY stage (r=–0.645, P=0.007), and patients with DED have lower BRs (12.14±2.54 *vs.* 9.0±2.06, P=0.031). In contrast, Fitzpatrick *et al.* could not find an association between BR and disease severity (53).

In addition, increased inflammatory cytokines have been detected in the tear film and conjunctival epithelium produced by the conjunctival and lacrimal gland, in accordance with the chronic inflammatory process in DED (54-57). Massingale *et al.* (18) analyzed cytokines in tear samples of seven subjects with DED and seven normal controls. They reported that all cytokines were increased in the tears of DED subjects compared with controls. In our study, IL-1β, IL-6 and IL-10 tear levels were higher in PD subjects than in controls, but this difference was not statistically significant probably due to a small sample size. Also, there was no evidence for a relationship between IL-1β, IL-6, IL-10, IL-12p70 and TNF-α tear concentration and clinical characteristics in PD subjects. Our study described that IL-8 was decreased in PD and presented association with the HY stage (r=0.523, P=0.034).

Interestingly, this study showed significant differences among patients with DED in PD regarding their BR and HY stage. These findings are consistent with the hypothesis that PD patients developed DED due to lower BY and subsequently increased pro-inflammatory cytokines.

Our study has several limitations, the sample size was too low, and further studies with larger numbers of patients were needed to confirm our findings. In addition, we used the Human Inflammatory Cytokine Kit to evaluate tear cytokines, while other reports in the literature used multiplex immunobead assay. Although we did not measure IL-4 and IFN-γ, it has been reported they are increased in serum of PD patients and linked to neuroinflammation. Moreover, a comparison between cytokines levels in serum and tears was not carried out. As mentioned before, our patient samples did not reflect the different stages of the disease.

Conclusions

PD patients have higher levels of TNF-α tears than age- and sex-matched HC. IL-8 in tears may be involved in the disease severity and DED in PD. Also relevant, the mechanisms behind the DED in PD seem to be related to BR and the severity of the disease. These findings should be confirmed in a larger group of patients.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://aes.amegroups.com/article/view/10.21037/aes-22-70/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://aes.amegroups.com/article/view/10.21037/aes-22-70/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional review board and local ethics Committee of the National Institute of Neurology and Neurosurgery, Mexico City, Mexico (No. 162-19). Informed consent was obtained from all individual participants.

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