Kynurenine pathway can be a potential biomarker of fatigue in primary Sjögren's syndrome

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Abstract

Objective

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease with low quality of life caused by various constitutional symptoms and glandular dysfunction. Although fatigue is one of the most frequent symptoms in pSS, its aetiology or biomarkers are poorly elucidated. We investigated potential relationship between severity of fatigue and the kynurenine pathway in pSS.

Methods

Clinical data and blood samples of 81 patients were obtained from a prospective cohort for pSS and compared with age- and sex-matched healthy controls (HC). Severity of fatigue was defined according to the fatigue domain scores in the ESSPRI. Potential biomarkers related to the kynurenine pathway were determined using ELISA.

Results

Of the total, 44 patients were defined as the "severe fatigue (ESSPRI fatigue ≥ 5)" group, whereas 37 as the "less fatigue (ESSPRI fatigue <5)". Serum tryptophan levels in the severe fatigue group were significantly lower while those of kynurenine were higher than in the others. Serum interferon gamma, IDO1, and quinolinic acid levels were mostly higher in the less fatigue group. Kynurenine/tryptophan ratios were distinctly higher in the severe fatigue group than both HC and the less fatigue group ($p<0.001$). This ratio showed a strong degree of positive correlation ($r=0.624$, $p<0.001$) with severity of fatigue in pSS while the other markers showed fair degrees of correlation.

Conclusion

Serum markers related to the kynurenine pathway, especially the kynurenine/tryptophan ratio, may be associated with severity of fatigue in pSS. These results can provide guidance for further investigations on fatigue in pSS.

Key words

Sjögren's syndrome, fatigue, biomarkers, tryptophan, kynurenine

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease that mainly causes inflammation of the exocrine glands, such as the lacrimal and salivary glands, by lymphocytic infiltration, resulting in glandular dysfunction (1). In addition to the glandular symptoms, various constitutional and extraglandular symptoms may also be present in most patients with pSS (2). Because of its wide range and chronicity of disease-related symptoms, the quality of life of patients with pSS usually deteriorates with disease progression (3, 4). Fatigue is one of the most frequent constitutional symptoms of pSS patients; about 70% of pSS patients suffer from fatigue according to the reported studies, which is much higher than that in heathy people (5). Although fatigue is regarded as a state of feeling tired physically and mentally, it is difficult to define and encompasses multifactorial aspects (6). Many studies have suggested associations between the degree of fatigue and poor quality of life in pSS patients (7, 8). However, the pathophysiological mechanisms or biomarkers of fatigue in pSS have not been elucidated until now.

Recently, the kynurenine pathway, which is a main tryptophan-metabolising pathway, has been suggested as a mechanism contributing to various neurological morbidities, including fatigue (9, 10). Tryptophan is one of the essential amino acids and acts as a precursor to various neurotransmitters and bioactive metabolites through its metabolising pathways such as the kynurenine pathway and the serotonin pathway (11). Although tryptophan 2,3-dioxygenase in the liver mainly participates in the first step of metabolising tryptophan in the homeostatic state, interferon gamma (IFN-γ) can be elevated in inflammatory conditions and induce expression of indoleamine-2,3-dioxygenase 1 (IDO1), which is another enzyme that metabolises tryptophan to kynurenine (12). Then, kynurenine can be further metabolised into two distinct metabolites, quinolinic acid and kynurenic acid. Because these metabolites bind to N-methyl-D-aspartate receptors in the brain, they can be neuroprotective or neurotoxic depending on their activities at receptors (11). Previously, kynurenine and quinolinic acid have been regarded as anti-inflammatory factors that increase the activities of regulatory T cells (13); however, recent studies have postulated that such kynurenine metabolites called "kynurenines" can induce local inflammation in the central nervous system (14). In fact, kynurenine metabolites are elevated in some medical conditions, such as chronic fatigue syndrome and fibromyalgia, compared to the healthy population (15). Several studies have reported that IDO1 activity, represented by the ratio of kynurenine to tryptophan, and kynurenine metabolites are increased and correlated with fatigue in patients with systemic lupus erythematosus and rheumatoid arthritis (16, 17).

IFN-γ activation has emerged as one of the mechanisms involved in the pathogenesis of pSS (12). Therefore, consequent activation of IDO1 and the kynurenine pathway is expected in patients with pSS. Although some studies have reported increased circulating levels of kynurenine metabolites in pSS patients (18), their relationships with severity of fatigue have not been explored. Herein, we performed a study aimed at investigating whether the kynurenine pathway is activated and shows any association with the degree of fatigue using clinical samples extracted from our prospective cohort database for pSS.

Materials and methods

Target population

All subjects in this study were selected from participants in the Korean Initiative of primary Sjögren's syndrome (KISS) cohort, which is a nation-wide prospective database containing clinical information and samples from pSS patients in Korea. Information on the cohort is described in detail elsewhere (19). Informed consent was obtained from all participants in the cohort according to the principles of the Declaration of Helsinki. All studies related to this cohort, including the present study, have been approved by the Institutional Review Board of Seoul St. Mary's Hospital of the Catholic University of Korea (approval number: KC13ON-

MI0646). We chose target subjects who met the 2016 ACR/EULAR classification criteria for pSS considering the availability of serum samples and clinical information (20). Patients previously diagnosed with comorbidities, such as fibromyalgia, chronic fatigue syndrome and mood disorders were excluded. Finally, data from 81 patients were selected from the cohort database as well as age- and sex-matched with healthy subjects as controls.

Clinical variables

All clinical data were extracted from the KISS cohort database. The demographic information, medication status, secretory functions measured by Schirmer I test, ocular staining scores, and unstimulated salivary flow, as well as serological profiles such as positivity for anti-Ro antibody, anti-La antibody, and rheumatoid factor were included in the analyses. Systemic disease activities and disease-related symptom severities were measured by the EULAR Sjögren's syndrome disease activity index (ESSDAI, range 0–123) (21), EULAR Sjögren's syndrome patient-reported index (ESSPRI, range 0–10) (22), and visual analogue scale (VAS, range 0–100) assessed by patients and physicians. The disease-related quality of life was measured as per the EuroQol (EQ) -5 dimensions (5D) time trade-off (TTO) values (range 0–1.000) (23) and EQ VAS (range 0–100). The presence of extraglandular manifestations (EGMs) was defined in case there was any presence of items included in Supplementary Table SI. The severity of fatigue was determined based on scores of the fatigue domain in ESSPRI. Patients with "severe fatigue" were defined based on a score of fatigue domain in ESSPRI ≥5, whereas patients were considered to have "less fatigue" if their scores were <5 (24).

Measurement of biomarker candidates

All serum samples used in the present study were acquired at the time of clinical assessment. The circulating levels of cytokines (IFN-γ), enzymes (IDO1), tryptophan, and their metabolites (L-kynurenine, quinolinic acid) in the sera of pSS patients as well as healthy controls (HC) were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (IDO1, tryptophan, L-kynurenine, and quinolinic acid: MyBioSource, San Diego, CA, USA; interferon gamma: R&D Systems, Inc., Minneapolis, MN, USA).

Table I. Clinical characteristics of the enrolled patients with primary Sjögren's syndrome.

	pSS, n=81		
	ESSPRI fatigue domain <5 n=37	ESSPRI fatigue domain ≥5 n=4	*p*-value
Age, years	54 (49–59)	51 (45–61)	0.372
Sex, female	36 (97.3)	43 (97.7)	> 0.999
Body mass index, kg/m^2	22.7 (22.0–24.2)	22.5 (20.2–24.9)	0.322
Disease duration, months	15 (1–53)	2 (0–38)	0.113
Medication status			
Methotrexate	0 (0)	1 (2.3)	> 0.999
Hydroxychloroquine	25 (67.6)	26 (59.1)	0.493
Corticosteroid	13 (35.1)	18 (40.9)	0.651
Pilocarpine	32 (86.5)	35 (79.5)	0.558
Secretory functions			
Unstimulated salivary flow ≤0.1 mL/min	32 (86.5)	33 (75.0)	0.196
Schirmer I test ≤5 mm/5 min	30/34 (88.2)	34/42 (81.0)	0.387
Ocular staining score ≥5	13/32 (40.6)	21/40 (52.5)	0.316
Serological profiles			
Anti-Ro antibody positivity	32 (86.5)	37 (84.1)	0.762
Anti-La antibody positivity	17 (45.9)	26 (59.1)	0.238
Rheumatoid factor positivity	27/36 (75.0)	26/41 (63.4)	0.273
ESSDAI total score	4 (0–7)	3 (1–7)	0.596
ESSPRI overall score	3.7 (2.2–4.5)	6.3 (5.1–7.0)	< 0.001
ESSPRI dryness domain	7 (5–8)	8 (6–9)	0.006
ESSPRI fatigue domain	2 (0–3)	7 (6–8)	< 0.001
ESSPRI pain domain	2 (0–3)	5 (2–7)	0.003
Presence of EGM	24 (64.9)	29 (65.9)	0.808
VAS for PtGA	54 (30–75)	77 (62–82)	0.001
VAS for PhGA	34 (15–45)	35 (11–50)	0.604
EQ-5D TTO value	0.89 (0.81–0.93)	0.77 (0.71–0.89)	0.005
EQ VAS	75 (64–85)	60 (50–75)	0.006

All data are expressed as n (%) or median (interquartile range).
pSS: primary Sjogren's syndrome; ESSPRI: EULAR patient-reported index; ESSDAI: EULAR Sjögren's syndrome disease activity index; EGM: extraglandular manifestation; VAS: visual analog scale; PtGA: patient's global assessment; PhGA: physician's global assessment; EQ-5D TTO: EuroQol-5 dimensions time trade-off.

Statistical analysis

Continuous variables were expressed in terms of the median and interquartile range before analysis using the Mann-Whitney U-test; the categorical variables were expressed as numbers (%) and analysed using the Chi-square test or Fisher's exact test. Correlations between the biomarker candidates and clinical variables were analysed using Spearman's rank correlation coefficient test. Statistical significance was determined based on *p*-value <0.05. The analyses were performed using IBM-SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL, USA), and all related figures were plotted using GraphPad Prism version 8.0 (GraphPad, San Diego, CA, USA).

Results

Clinical characteristics of target population

Among the 81 pSS patients, 37 were designated as the "less fatigue" group while the remaining 44 were classified as the "severe fatigue" group. The two groups showed similar ages, sex proportions, disease durations, and medication statuses (Table I). The secretory functions, serological profiles, ESSDAI scores, and presence of EGMs were not different between the two groups. The median values of the ESSPRI fatigue domain in the severe fatigue and less fatigue groups were 7 and 2, respectively. The severe fatigue group presented higher scores of dryness and pain in ESSPRI as well as

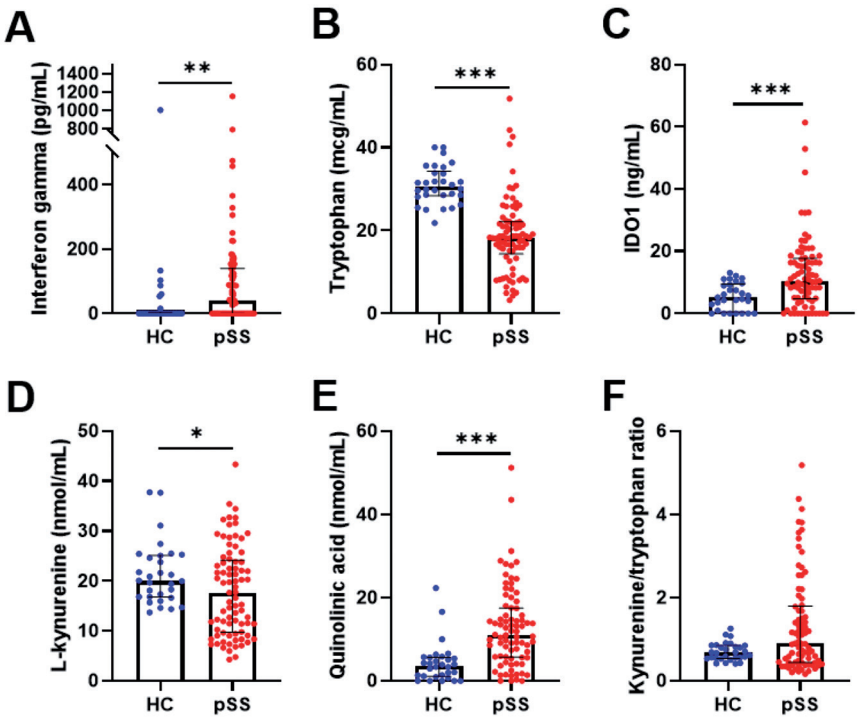

Fig. 1. Serum levels of tryptophan, its metabolites, and related enzymatic activities in patients with primary Sjögren's syndrome (pSS) and healthy controls (HC). (**A**) Interferon gamma; (**B**) tryptophan; (**C**) indoleamine-2,3-dioxygenase 1 (IDO1); (**D**) L-kynurenine; (**E**) quinolinic acid; (**F**) ratio of L-kynurenine to tryptophan. All data were measured using enzyme-linked immunosorbent assay. The bars indicate the median and interquartile range.
*p<0.05, **p<0.01, ***p<0.001.

ESSPRI total scores compared to the less fatigue group. VAS values for the disease symptom severity assessed by the patients themselves were higher in the severe fatigue group than the less fatigue group, whereas VAS values for disease activity assessed by the physicians showed no differences between the two groups. Both EQ-5D TTO and EQ VAS values were significantly lower in the severe fatigue group than the less fatigue group. Such results on patient-reported outcomes suggest that patients with higher degrees of fatigue considered their disease more severe and quality of life lower.

Comparison of serum levels of potential biomarkers between pSS and HC

Considering that previous studies have reported increased IFN-γ levels and tryptophan-metabolising activities in pSS, we first assessed the differences in serum levels of potential biomarkers related to such pathways between the pSS patients (n=81) and HC (n=30) in our cohort. The circulating levels of IFN-γ were significantly higher in the sera of pSS patients than in HC (Fig. 1A). Serum levels of tryptophan were significantly lower while those of IDO1, which is a main activating enzyme for metabolising tryptophan in inflammatory conditions, were higher in pSS patients than HC (Fig. 1B and C). Serum levels of L-kynurenine, one of the main metabolites of IDO1-mediated tryptophan metabolism, were markedly lower whereas those of quinolinic acid, which is a more downstream metabolite than L-kynurenine in the tryptophan-metabolising pathway, were significantly higher in pSS patients than HC (Fig. 1D and E). The kynurenine/tryptophan ratio is thus regarded as a marker representing the enzymatic activity of IDO1 (25). In the present study, this ratio was not significantly different between the pSS patients and HC (Fig. 1F).

Comparison of serum levels of potential biomarkers based on the severity of fatigue

We compared the serum levels of potential biomarker candidates between pSS with severe fatigue (n=44) and less fatigue (n=37) as well as HC. Serum levels of IFN-γ were significantly higher only in the less fatigue pSS than HC (Fig. 2A). There were no differences between the severe fatigue pSS and HC. Circulating levels of tryptophan were the most decreased in sera from the severe fatigue group (Fig. 2B). As the degree of fatigue get more severed, serum levels of tryptophan tended to decrease. Serum levels of IDO1 were significantly increased only in less fatigue pSS than HC and severe fatigue pSS (Fig. 2C). As with IFN-γ, circulating IDO1 levels were not significantly different between HC and severe fatigue pSS. Interestingly, serum L-kynurenine levels were significantly lower in less fatigue pSS than HC and severe fatigue pSS (Fig. 2D). This finding suggests the reason for the previous results showing lower L-kynurenine levels in overall pSS patients than HC (Fig. 1D) despite the marginally higher L-kynurenine levels in severe fatigue pSS than HC (Fig. 2D). Serum levels of quinolinic acid in both less and severe fatigue groups were significantly higher than that in HC (Fig. 2E). Between the two pSS groups, the less fatigue group presented higher serum levels of quinolinic acid than the severe fatigue group. Notably, the kynurenine/tryptophan ratio was distinctly higher in the sera of severe fatigue pSS than HC and less fatigue pSS (Fig. 2F). Although the previous results suggested no differences in the serum kynurenine/tryptophan ratios between HC and overall pSS (Fig. 1F), this ratio showed the potential for distinguishing pSS patients with severe fatigue based on results of subsequent analyses dependent on the severity of fatigue (Fig. 2F).

Relation between degree of fatigue and serum levels of potential biomarkers

Next, we assessed the relationships between serum levels of IFN-γ, tryptophan metabolites, and related enzymatic activities and degree of fatigue represented by scores of the fatigue domain in ESSPRI in pSS patients (n=81). The circulating levels of IFN-γ did not correlate well with degree of

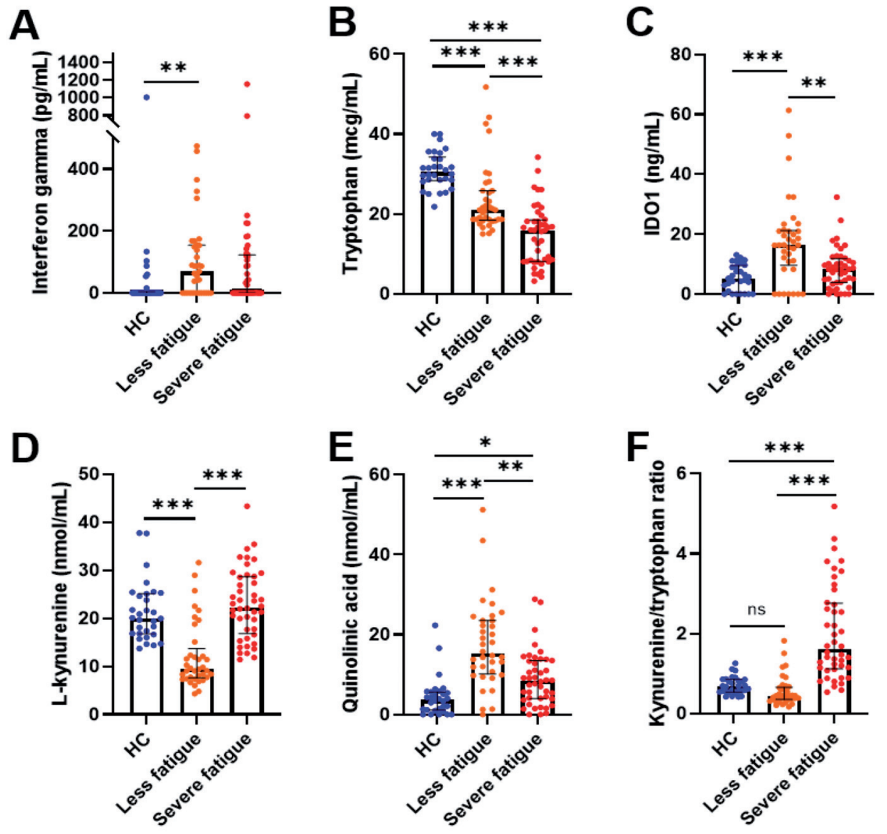

Fig. 2. Serum levels of tryptophan, its metabolites, and related enzymatic activities in patients with primary Sjögren's syndrome with less fatigue (ESSPRI fatigue domain <5), and severe fatigue (ESSPRI fatigue domain ≥5), and healthy controls (HC). (**A**) Interferon gamma; (**B**) tryptophan; (**C**) indoleamine-2,3-dioxygenase 1 (IDO1); (**D**) L-kynurenine; (**E**) quinolinic acid; (**F**) ratio of L-kynurenine to tryptophan.
All data were measured using enzyme-linked immunosorbent assay. The bars indicate the median and interquartile range.
* p<0.05, ** p<0.01, *** p<0.001.

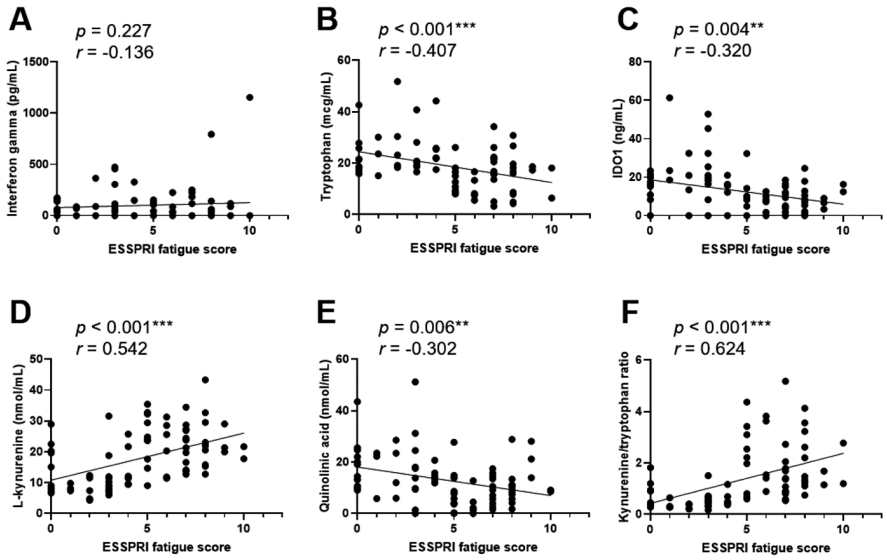

Fig. 3. Correlations between degree of fatigue and serum levels of (**A**) interferon gamma, (**B**) tryptophan, (**C**) indoleamine-2,3-dioxygenase 1 (IDO1), (**D**) L-kynurenine, (**E**) quinolinic acid, and (**F**) ratio of L-kynurenine to tryptophan. The degree of fatigue is defined as score of the fatigue domain in ESSPRI; 'r' indicates Spearman's correlation coefficient.
*p<0.05, ** p<0.01, *** p<0.001.

fatigue (Fig. 3A), whereas other potential biomarkers showed statistically significant correlations with degree of fatigue (Fig. 3B-F). Serum levels of tryptophan, IDO1, and quinolinic acid showed moderate negative correlations (Fig. 3B, C and E) while those of L-kynurenine and kynurenine/tryptophan ratio showed moderate-to-strong positive correlations with degree of fatigue (Fig. 3D and E). The kynurenine/tryptophan ratio showed the strongest correlation (r=0.624) with degree of fatigue (Fig. 3F).

Relation between disease-specific variables and serum levels of potential biomarkers

Then, we determined the relation between potential biomarkers and pSS-specific variables, such as serum positivity for anti-Ro/La antibodies, presence of any EGMs, and secretory functions represented by unstimulated salivary flow rates, Schirmer I test, and ocular staining scores. The serum levels of all the potential markers did not show significant differences depending on the positivity for anti-Ro/La antibodies and the presence of any EGMs (Table II). We also analysed correlations between parameters related to secretory functions and biomarkers. Although unstimulated salivary flow rates showed a weak negative correlation with IDO1, other analyses did not make statistical significance (Table II).

Relation between other clinical indices and serum levels of potential biomarkers

Lastly, we evaluated whether the serum levels of IFN-γ, tryptophan metabolites, and related enzymatic activities correlated with other clinical parameters, such as ESSDAI (systemic disease activity) and EQ-5D TTO values (disease-related quality of life), including the other domains and total scores of ESSPRI. Most parameters, such as ESSDAI, scores of pain domain and dryness domain in ESSPRI, and EQ-5D TTO values, were not correlated with potential biomarkers related to tryptophan metabolism (Table III). On the other hand, ESSPRI total scores

Table II. Relations between disease-specific variables and serum potential biomarkers of fatigue in primary Sjögren's syndrome.

	Anti-Ro antibody			Anti-La antibody			Extraglandular manifestations					
	Positive, n=69	Negative, n=12	*p*-value	Positive, n=43	Negative, n=38	*p*-value	Present, n=53	Absent, n=28	*p*-value	USFR†	Schirmer I test†	OSS†
Interferon gamma, pg/mL	26.2 (0–142.4)	0 (0–68.9)	0.372	61.5 (0–149.0)	0 (0–97.1)	0.183	38.9 (0–153.4)	59.2 (0–113.1)	0.740	-0.146	0.176	-0.203
Tryptophan, mcg/mL	18.3 (15.8–22.8)	15.3 (8.5–20.8)	0.223	18.2 (15.1–22.4)	18.5 (12.3–21.9)	0.931	17.5 (13.5–21.6)	18.7 (16.6–25.4)	0.271	-0.021	0.024	-0.036
IDO1, ng/mL	11.2 (5.1–18.2)	8.3 (0.5–15.4)	0.228	11.2 (7.2–18.6)	8.5 (3.1–16.7)	0.231	11.2 (5.1–17.0)	9.9 (4.1–19.8)	0.780	-0.242* (0.029)	0.203	0.033
L-kynurenine, nmol/mL	15.3 (9.5–22.8)	24.1 (12.9–29.9)	0.143	17.7 (10.3–22.6)	17.7 (9.1–27.1)	0.897	17.7 (9.7–24.2)	17.8 (8.8–25.3)	0.939	0.074	-0.085	0.010
Quinolinic acid, nmol/mL	12.0 (7.2–19.6)	5.6 (1.6–14.2)	0.071	9.9 (5.8–17.5)	13.4 (5.6–18.6)	0.483	11.0 (5.8–16.6)	12.2 (4.2–24.0)	0.564	-0.064	0.060	0.001
Kynurenine/tryptophan ratio	0.88 (0.44–1.60)	1.65 (0.73–2.96)	0.096	1.08 (0.54–1.54)	0.85 (0.43–2.18)	0.949	0.95 (0.45–1.80)	0.85 (0.45–1.86)	0.765	0.090	-0.044	0.042

All data are expressed as median (interquartile range).
†Data of these variables are expressed as Spearman's correlation coefficient (*p*-value, if significant).
IDO1: indoleamine-2,3-dioxygenase 1; USFR: unstimulated salivary flow rates; OSS: ocular staining score.
*$p<0.05$.

Table III. Correlations between clinical parameters and serum potential biomarkers of fatigue in primary Sjögren's syndrome.

	ESSDAI	ESSPRI pain domain	ESSPRI dryness domain	ESSPRI total score	EQ-5D TTO value
Interferon gamma	0.316** (0.005)	-0.195	-0.064	-0.196	0.058
Tryptophan	0.034	-0.094	-0.121	-0.286** (0.009)	0.050
IDO1	0.154	-0.146	-0.038	-0.248* (0.025)	0.101
L-kynurenine	0.086	0.208	0.180	0.456*** (< 0.001)	-0.147
Quinolinic acid	-0.069	0.114	-0.052	-0.106	-0.033
Kynurenine/tryptophan ratio	0.045	0.175	0.179	0.463*** (< 0.001)	-0.139

All data are expressed as Spearman's correlation coefficient (*p*-value, if significant).
ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR patient-reported index; EQ-5D TTO: EuroQol-5 dimensions time trade-off; IDO1: indoleamine-2,3-dioxygenase 1.
*$p<0.05$, **$p<0.01$, ***$p<0.001$.

showed weak-to-moderate correlations with serum levels of tryptophan, IDO1, L-kynurenine, and the kynurenine/tryptophan ratio, although the degree of correlation was weaker than that of the ESSPRI fatigue score. These findings show tendencies that the overall ESSPRI scores of participants in the present study were reflected by scores of the fatigue domain in ESSPRI. Interestingly, the circulating level of IFN-γ showed a weak positive correlation with ESSDAI, which reflects systemic disease activities in pSS. This finding is consistent with the results of a previous study that reported increased interferon-related signals in pSS patients (26).

Discussion

In the present study, we assessed the expression levels of serum markers related to the kynurenine pathway, which metabolised tryptophan in pSS patients. The kynurenine pathway was activated in pSS patients compared to HC. Serum tryptophan and kynurenine levels were different in patients with more severe fatigue than in others. The kynurenine to tryptophan ratio was the most distinct marker for distinguishing pSS patients with more fatigue from those with less fatigue and HC. This ratio also showed the strongest correlation with severity of fatigue among the other potential biomarkers related to the kynurenine pathway. On the other hand, these kynurenine-pathway-related markers were not directly correlated with clinical parameters related to systemic disease activities or disease-associated quality of life.

Fatigue is closely related to the daily life of pSS patients. Previous studies reported that fatigue is one of the main predictors of low quality of life as well as high disease activity and can cause impaired functional status in pSS (7, 8). Such findings were replicated in the present study. Patients with more severe fatigue suffered from lower quality of life according to the patient-reported outcomes represented by EQ-5D TTO values (Table I). Therefore, identification of factors contributing to fatigue was attempted in past studies. Karageorgas *et al*. investigated the clinical or laboratory parameters associated with fatigue in pSS patients (27). In a multivariate analysis, only the psychometric aspects such as depression and fibromyalgia were relevant to fatigue, whereas the biological markers such as mRNA expression levels of IDO1 as well as type I and II interferon-induced genes were not relevant. Although we directly measured the serum levels of IFN-γ and IDO1, they could not distinguish the more fatigued patients from others (Fig. 2A and C), as in the previous study by the Greek group (27).

Instead, the actual tryptophan-metabolising activity of IDO1, represented by the serum kynurenine to tryptophan ratio, was more relevant to the severity of fatigue according to the present study. This finding implies that the actual enzymatic activities of IDO1 are more important for the severity of fatigue in pSS than the circulating levels of IDO1, which can fluctuate throughout disease progress.

When pSS patients were classified according to degree of fatigue, those with less fatigue presented distinct biomarker profiles than both HC and severe fatigue group in the present study. In the less fatigue patients, the serum levels of kynurenine were remarkably lower compared to those of HC (Fig. 2D), whereas those of quinolinic acid were significantly higher than the severe fatigue group (Fig. 2E). Although the serum tryptophan levels gradually decreased as the degree of fatigue increased in severity (Fig. 2B), such findings allow us to postulate that the less fatigue pSS patients are not a mere intermediate state between HC and severe fatigue patients; instead, they are another distinct subset of pSS unlike the patients with more fatigue. A recent proteomic study in the Netherlands suggested several differentially expressed proteins between the fatigued and non-fatigued pSS patients (28). Although a mechanistic explanation was absent, the upregulated proteins in fatigued patients, such as neuroactive- synaptosomal-associated protein 25, alpha enolase, and ubiquitin carboxyl-terminal hydrolase isozyme L1, were suggested as "fatigue signatures". According to these findings, we must consider a stratified approach for fatigued pSS patients because distinct pathophysiological mechanisms may be involved. Furthermore, although we mainly focused on the kynurenine pathway in the present study, novel therapeutic targets should be investigated to manage fatigue in pSS patients considering such results.

Increased type I and type II interferon signatures in pSS have been reported in various studies in the past (12, 26, 29). However, many novel therapeutics targeting interferons have not shown satisfactory results in clinical evaluations (30). Furthermore, many clinical trials reported some discrepancies in the results between patient-reported outcomes involving severity of fatigue, such as the ESSPRI, and other clinical indices like the ESSDAI and biological parameters (31). A recent study using nuclease therapy showed improvement of fatigue whereas ESSDAI and expressions of interferon-inducible genes remained unimproved (32). In the present study, although the serum levels of IFN-γ increased in pSS patients over those of HC (Fig. 1A), they did not represent severe fatigue or correlate with the degree of fatigue (Fig. 2A and 3A). Instead, IFN-γ was correlated with the systemic disease activity of pSS (Table III). The results of clinical trials and findings of this study suggest that direct targeting interferons, regardless of type I or type II, may not be effective for managing fatigue in pSS patients. Considering the results of the present study, targeting the IDO1 activity may be a more promising therapeutic approach than directly targeting interferons.

The present study has several limitations as follows. First, the degree of fatigue measurement was determined only based on the ESSPRI scores. The severity of fatigue can be semi-quantified by several tools, such as the Fatigue Severity Score, Functional Assessment of Cancer Therapy Scale-fatigue, Profile of Fatigue and Discomfort-Sicca Symptoms Inventory, and Medical Outcomes Study Short-Form. However, because the present study was performed on pre-existing cohort data, such measurements were lacking. Second, the sample sizes were too small to maximise statistical significance as well as determine sensitivity and specificity of severe fatigue. Third, because only the serum samples were available for this study, the measurement of various interferon-inducible genes or other metabolites were lacking. All these drawbacks can be addressed in further studies with prospective settings involving larger numbers of participants and samples.

Despite the above limitations, this study is a pioneering work reporting novel biomarkers that rigorously correlated with the severity of fatigue in pSS patients. The kynurenine pathway, especially IDO1 activity, is significantly associated with severe fatigue in pSS. The findings of the present study are therefore expected to provide critical guidance for future studies investigating the mechanisms and therapeutic targets of fatigue in pSS.

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