Elevated Kynurenine Levels in Patients with Primary Sjögren's Syndrome

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Abbreviations: Trp, tryptophan; pSS, primary Sjögren's syndrome; Kyn, kynurenine; sSS, secondary SS; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; IDO, indoleamine 2,3-dioxygenase; EULAR, European League Against Rheumatism; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR SS Patient Reported Index; LC-MS/MS, liquid chromatography with tandem mass spectrometry; SSc, systemic sclerosis; CRP, C-reactive protein; KP, kynurenine pathway; AS, ankylosing spondylitis.

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ABSTRACT

Objective: We aimed to investigate the plasma levels of tryptophan (Trp) and its metabolites in patients with primary Sjögren's syndrome (pSS).

Methods: The study included 34 pSS patients and 42 healthy individuals, and serum Trp and kynurenine (Kyn) concentrations were measured by liquid chromatography with tandem mass spectrometry. Trp degradation was predicted using the ratio of Kyn and Trp concentrations (Kyn/Trp).

Results: In our study, the mean serum Trp concentration was found to be considerably lower in the pSS group than in the control group ($P = .001$). The levels of Kyn ($P = .019$) and the Kyn/Trp ratio ($P < .001$) were significantly higher in the pSS group than in the control group. The Kyn/Trp ratio was negatively correlated with C-reactive protein ($r = -0.369$, $P = .032$).

Conclusion: We found that Kyn pathway metabolism was altered in patients with pSS. This suggests that Trp metabolism may be closely linked to the disease pathogenesis of pSS.

Primary Sjögren's syndrome (pSS) is a chronic systemic autoimmune illness characterized by salivary and lacrimal gland involvement, resulting in keratoconjunctivitis sicca and xerostomia, as well as various extraglandular signs such as severe fatigue, vasculitis, and multiorgan involvement.[1] Secondary Sjögren's syndrome (sSS), which occurs as a result of other autoimmune illnesses, is distinguished from pSS[2]; sSS is commonly found in connective tissue diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and it is less commonly found in multiple sclerosis, autoimmune hepatitis, and thyroiditis.[3] Sjögren's syndrome predominantly affects women, and the incidence of the disease ranges from 3 to 11 cases per 100,000 population,[4,5] with a prevalence ranging from 0.01% to 0.72%.[6]

pSS is a disease whose etiology is not yet fully understood, as in most autoimmune diseases, where genetic, epigenetic, and environmental factors are hypothesized to play into the pathogenesis of the disease. pSS has a complicated pathophysiology that is multifaceted and linked to a number of genetic, immunological, environmental, and hormonal risk factors.[7] The disease course can be divided into several phases due to its complexity: an initiation phase triggered by exogenous and endogenous causes, dysregulation of salivary gland epithelial cells, immune system activation, and chronic inflammation stimulated by B cell hyperactivity.[8] Both innate and acquired immunity play a role in the pathogenesis of pSS, each constituting a multistep process that leads to illness onset and persistence. Persistent B-cell activation and the proliferation of Th1 and Th17 cells contribute to the progression of the disease.[9] Nevertheless, the exhaustive mechanism of disease progression in the salivary and lacrimal glands is still unknown.[10]

Serotonin and kynurenines (Kyn) are the indispensable amino acid tryptophan (Trp) metabolites, and the Kyn pathway is the main pathway of Trp metabolism, responsible for approximately 90% of its catabolism. The rate-limiting enzyme in this Trp catabolism pathway is indoleamine 2,3-dioxygenase (IDO), which converts Trp to Kyn.[11] It is thought that the catabolism of Trp via Kyn plays an important immunosuppressive role.[12,13] It has been found that blood Trp concentrations are decreased in RA patients,[14] various cancers,[15] and other autoimmune diseases such as antineutrophil cytoplasmic antibody-associated vasculitis.[16] The IDO expression is induced by immune mediators, particularly interferon.[17] Although IDO expression is usually quite low, it can be significantly upregulated in response to infection and inflammation.[18] The Kyn pathway produces an immunosuppressive effect by simultaneously activating Treg cells and decreasing effector T cell responses.[19] The IDO

plays a substantial role in sustaining peripheral immunological tolerance.[20] Increased Kyn concentration is common in many diseases, such as RA,[21] SLE,[22] and Huntington disease.[23] Also, Kyn and its metabolites are biologically active. For instance, in the immune system, Kyn and its metabolites play a role in suppressing the immune system.[13] Recently, there has been increasing interest in the role of Kyn in the regulation and maintenance of immune homeostasis. However, the precise role of IDO and the regulatory Kyn pathway in autoimmunity remains unclear.

In this study, we investigated levels of Trp and its metabolites in the Kyn pathway in patients with pSS and in healthy controls. Also, through the relationship between Trp metabolites, laboratory parameters, and disease activity was evaluated in patients with pSS.

Materials and Methods

Patients

This study was conducted in Ankara Yıldırım Beyazıt University Medical Faculty Atatürk Training and Research Hospital and Ankara City Hospital. Thirty-four pSS patients who were diagnosed according to the 2016 classification criteria of the American College of Rheumatology/European League Against Rheumatism (EULAR)[24] and 42 healthy volunteers were recruited into the study. The EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI)[25] and the EULAR SS Patient Reported Index (ESSPRI)[26] were used to assess disease activity. Healthy subjects who did not have an autoimmune condition and malignancy were included as controls. At the time of enrollment, all study participants were checked to ensure that they were free of symptoms of underlying viral infections. The study was approved by the ethics committee decision of Ankara Yıldırım Beyazıt University Medical Faculty Local Ethics Committee and written informed consent was obtained from all subjects.

Analysis and Determination of Tryptophan and Kynurenine Concentrations

Following the approval of the ethics committee, peripheral venous blood samples from all individuals included in the study under fasting (12 hours) were taken into tubes for blood testing. Plasma and serum samples were obtained by centrifugation for 10 minutes at 3000*g* and were stored at −80°C until day of analysis. Parameters were measured on the same day.

Trp and Kyn were measured in plasma samples using liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described.[27] Trp degradation was determined using the ratio of Kyn and Trp concentrations. This ratio shows IDO enzyme activity.[28] The Kyn/Trp ratio was calculated in μmol/mmol.

Statistical Analysis

Continuous variables showing normal distribution were analyzed by Kolmogorov-Smirnov test. Independent samples *t*-test and Mann-Whitney *U* test were performed to evaluate whether there was a statistically significant difference between patient and control groups. Correlations between groups were evaluated by Spearman rank correlation analysis. Statistical results of parametric and nonparametric variables are expressed as mean ± standard deviation and median (interquartile range [IQR]), respectively. Statistical analyses were performed using the SPSS version 21.0 package program, and $P < .05$ was accepted as statistically significant.

Results

The study comprised a total of 34 pSS patients and 42 healthy controls. The patient and control groups are similar with regard to age, gender, body mass index, smoking, and comorbidity. Characteristics of patients with pSS and controls are reported in **TABLE 1**.

TABLE 1. Characteristics of Patients with Primary Sjögren's Syndrome and Controls[a]

Characteristics	Sjögren's Syndrome (n = 34)	Control (n = 42)	*P*
Age (y), mean ± SD (minimum–maximum)	53 ± 11 (25–71)	54 ± 11 (27–67)	.612[b]
BMI (kg/m^2), mean ± SD (minimum–maximum)	29 ± 4 (23–41)	28 ± 3 (20–35)	.513[b]
Sex	32 (94.1)	39 (92.9)	.601[c]
Female			
Male	2 (5.9)	3 (7.1)	
Smoking	28 (82.4)	36 (85.7)	.737[d]
None	1 (2.9)	2 (4.8)	
Ex-smoker	5 (14.7)	4 (9.5)	
Active			
DM	3 (8.8)	3 (7.1)	.556[c]
HT	10 (29.4)	13 (31.0)	.884[d]
CAD	4 (11.8)	6 (14.3)	.511[c]
CKD	1 (2.9)	2 (4.8)	.580[c]
COPD	1 (2.9)	2 (4.8)	1.000[c]
IBD	1 (2.9)	2 (4.8)	1.000[c]

BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HT, Hashimoto thyroiditis; IBD, inflammatory bowel disease.
[a]*Data are given as No. (%) unless indicated otherwise.*
[b]*Independent* t *test.*
[c]*Fisher exact test.*
[d]*Pearson's* χ^2 *test.*

Median (IQR) duration of disease was 84 (30–120) months. The most common clinical findings were keratoconjunctivitis sicca (n = 34, 100%), xerostomia (n = 33, 97.1%), an abnormal Schirmer's test (n = 26, 76.5%), positive minor salivary gland biopsy (n = 28, 82.4%), synovitis (n = 23, 67.6%), fibromyalgia (n = 15, 44.1%), and fatigue (n = 26, 76.5%). Most of the patient results showed antinuclear antibody (ANA) positivity (n = 27, 79.4%) followed by anti-SS-A/Ro positivity (n = 20, 58.8%) and rheumatoid factor positivity (n = 25, 75%). The majority of patients were receiving hydroxychloroquine treatment (n = 30, 88.2%) followed by prednisolone treatment (n = 13, 38.2%). Clinical characteristics of the patients with pSS are shown in **TABLE 2**.

When the groups were compared in terms of Kyn pathway results, there were significant differences between Kyn (P = .019), Trp (P = .001), and the Kyn/Trp ratio (P < .001) between the patient and control groups. Level of Kyn and Kyn/Trp ratio was significantly higher in the pSS group whereas tryptophan was significantly lower (**TABLE 3**).

Trp concentration was positively correlated with albumin (r = 0.384, P = .025) and hemoglobin (P = .396, r = 0.020). Kyn/Trp ratio was negatively correlated with C-reactive protein (CRP) (r = –0.369, P = .032). Correlations between Kyn pathway results and clinical parameters in patients with pSS is reported in **TABLE 4**. In the results of linear regression analysis, hemoglobin explained 15% of Trp concentration (R^2 = 0.15, F = 6.018, P = .020). Also, glucose explained 15% of Kyn concentration (R^2 = 0.15, F = 5.869, P = .021) (**TABLE 5**).

No significant relation was found between the Kyn pathway and the presence of fibromyalgia, SS-A positivity and SS-A negativity, or prednisolone use in patients with pSS (P > .05) (**TABLE 6**).

TABLE 2. Clinical Characteristics of Patients with Sjögren's Syndrome

Characteristic (n = 34)	Result
Duration of disease, mo, median (IQR)	84 (30–120)
Disease activity scores, median (IQR)	
ESSDAI	2 (2–4)
Doctor global assessment	4 (3–4)
Patient global assessment	5 (3–6)
ESSPRI	4 (3–4)
Dryness	4 (3-5)
Fatigue	4 (3-5)
Pain	4 (3-5)
Clinical findings, No. (%)	
Ocular symptom	34 (100)
Oral symptom	33 (97.1)
Schirmer test right	26 (76.5)
Schirmer test left	26 (76.5)
Minor salivary gland biopsy	28 (82.4)
Synovitis	23 (67.6)
Leukopenia	4 (11.8)
Interstitial lung disease	1 (2.9)
Fibromyalgia	15 (44.1)
Fatigue	26 (76.5)
Laboratory signs, mean (SD) (95% CI)	
WBC	6319 (2209) (5548–7089)
HGB	12.8 (1.4) (12.3–13.3)
PLT	258,324 (73,422) (232,705–283,941)
Glucose	89 (12) (84.9–93.6)
Creatinine, median (IQR)	1 (1–1)
AST, median (IQR)	22 (15–24)
ALT, median (IQR)	19 (15–21)
ALP, median (IQR)	72 (47–80)
GGT, median (IQR)	16 (11–20)
Albumin, mean (SD) (%95 CI)	44 (3) (43.3–45.4)
Total protein, mean (SD) (%95 CI)	73 (5) (71.1–74.7)
Sedimentation, median (IQR)	17 (5–19)
CRP, median (IQR)	3 (1–5)
Serological findings, No. (%)	
ANA positivity	27 (79.4)
RF positivity	25 (73.5)
Anti-CCP positivity	4 (11.8)
SS-A positivity	20 (58.8)
SS-B positivity	6 (17.6)
Treatment	
Prednisolone, No. (%)	13 (38.2)
Prednisolone dose, mg/d, median (IQR)	4 (2–4)
Hydroxychloroquine, No. (%)	30 (88.2)
Methotrexate, No. (%)	6 (18.2)
Azathioprin, No. (%)	1 (2.9)

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CCP, cyclic citrullinated peptide; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; GGT, gamma-glutamyl transferase; HGB, hemoglobin; IQR, interquartile range; PLT, platelets.

Discussion

In this study, we report higher levels of the Trp metabolite Kyn and an increased Kyn/Trp ratio in patients with pSS compared with controls. We found that levels of Trp were lower in pSS as measured by LC-MS/MS. Although Trp was negatively correlated with the physician global assessment, a positive correlation was observed with hemoglobin and albumin. The Kyn/Trp ratio showed a negative correlation with CRP. No significant relationship was found between the Kyn pathway metabolites and fibromyalgia, SS-A positivity, or prednisolone use in patients with pSS.

Trp, the rarest indispensable amino acid, is a precursor for protein synthesis and the manufacture of many chemicals involved in basic biological processes. The Kyn pathway metabolizes the vast majority of Trp (90%), with the serotonin pathway converting the remaining 1% to serotonin and melatonin.[29,30] The IDO expression is upregulated locally and systemically in numerous tissues by immune activation and inflammation, and IDO reduces the concentration of Trp in the milieu of inflammatory cells.[31,32] The IDO activity can inhibit a potentially harmful autoimmune response, induce peripheral tolerance, and minimize chronic immunological activation in T cells, which are particularly susceptible to Trp depletion.[33,34] Overexpression of IDO has been observed in patients with SLE, pSS, and sepsis.[35] Serum Trp levels are higher in healthy women than in women diagnosed with pSS. However, patients with pSS have higher Kyn levels and a higher Kyn/Trp ratio than healthy women and patients with non-SS sicca. The same findings were made in men with pSS, indicating that the IDO enzyme activity in the kynurenine pathway (KP) is enhanced.[34,36] In the metabolic profile comparison of SLE with pSS and systemic sclerosis (SSc), decreased levels of

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TABLE 3. Comparison of the Kynurenine Pathway Results of Patients with Sjögren's Syndrome and Controls

	Sjögren's Syndrome (n = 34), Median (IQR)	Control Group (n = 42), Median (IQR)	P[a]
Kynurenine (ng/mL)	485 (378–601)	386 (356–496)	**.019**
Tryptophan (ng/mL)	10,660 (9160–12,282)	12,258 (11,442–14,711)	**.001**
Kynurenine/tryptophan ratio (%)	4 (3–6)	3 (3–4)	**<.001**

[a]*Mann-Whitney* U *test.*

TABLE 4. Correlation between Kynurenine Pathway Results and Clinical Parameters in Patients with Primary Sjögren's Syndrome[a]

	Kynurenine, *r*	Tryptophan, *r*	Kynurenine/Tryptophan Ratio, *r*
Age	0	0	–0.08
BMI	–0.05	0.04	–0.11
Duration of disease	0.08	–0.06	0.05
Prednisolone dose	–0.08	0.1	–0.18
Laboratory signs			
WBC	0.13	–0.03	0.1
HGB	0.04	**0.39**[b]	–0.28
PLT	–0.24	0.01	–0.26
Glucose	**–0.39**[b]	0.02	–0.28
Creatinine	–0.17	–0.08	–0.03
AST	–0.07	0.17	–0.22
ALT	–0.17	0.19	–0.24
ALP	0.26	–0.05	0.08
GGT	0.1	0.28	–0.19
Albumin	–0.19	**0.38**[b]	–0.28
Total protein	–0.15	0.04	–0.19
Sedimentation	0.08	-0.16	0
CRP	–0.16	0.19	**–0.37**[b]
Disease activity scores			
ESSDAI	–0.29	0.07	–0.21
Doctor global assessment	–0.3	0.11	–0.26
Patient global assessment	–0.25	0.1	-0.19
ESSPRI	–0.23	0.11	–0.17
Dryness	–0.09	0.17	–0.11
Fatigue	–0.28	0.07	–0.17
Pain	–0.21	0.15	–0.24

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; GGT, gamma-glutamyl transferase; HGB, hemoglobin; PLT, platelets.
[a]*Spearman's correlation coefficients* (r) *above |0.3| are highlighted in bold.*
[b]P < *.05.*

tryptophan were seen in SLE patients compared to control groups.[37] In studies conducted on the role of IDO and Trp catabolism in RA in humans, it has been shown that Trp levels decrease and Kyn levels increase in a patient's sera.[21,38] When compared to healthy controls, persons with RA were shown to have lower Trp levels and greater Kyn levels.[14,39,40] These findings were verified in a mouse model of collagen-induced arthritis, where activation of the Trp pathway was revealed to be critical during the arthritis induction and resolution phases.[41] Similar to the literature, in this study we found that Kyn levels were higher, Kyn/Trp ratio increased, and Trp levels were lower in patients with pSS compared to the control group.

Increased IDO serum activity has previously been defined in patients with pSS and was associated with disease severity.[35] Patients with RA show a distribution of Trp metabolites in serum characterized by a high ratio of Kyn/Trp. In persons with RA, decreased Trp and increased Kyn have been linked to disease activity and clinical symptoms.[14,42] The IDO activity was increased in patients with SLE in a cross-sectional study.[43] Patients with active disease (SLE disease activity index ≥6) showed lower Trp levels than controls, but IDO levels of patients with SLE in remission were not different from those in controls. Trp metabolites Kyn and quinolinic acid were higher in patients with SLE compared to controls.[43] In a study evaluating serum levels of Kyn pathway

TABLE 5. Regression Analysis Results for Kynurenine and Tryptophan[a]

	Kynurenine				Tryptophan			
	Constant	B	*t*	*P*	Constant	B	*t*	*P*
Glucose	833.1	−3.9	−2.4	**<.001**	NA	NA	NA	NA
Hemoglobin	NA	NA	NA	NA	-397.6	866.2	2.4	**.02**

NA, not applicable.
[a]*Linear regression analysis.* P < *.05 is highlighted in bold.*

TABLE 6. Comparison of the Kynurenine Pathway Results for Fibromyalgia, SS-A, and Prednisolone in Patients with Sjögren's Syndrome

	Tryptophan (ng/mL), Median (IQR)	Kynurenine (ng/mL), Median (IQR)	Kynurenine/Tryptophan ratio (%), Median (IQR)
Control group (n = 42)	12,258 (11,442–14,711)	386 (356–496)	3 (3–4)
Patients with Sjögren's syndrome			
Fibromyalgia (n = 15)	10,609 (8527–18,319)	478 (317–611)	4 (3–6)
Nonfibromyalgia (n = 19)	10,839 (6842–13,934)	509 (315–670)	5 (3–14)
P value	.456	.456	.206
SS-A positivity (n = 20)	10,429 (6842–12,662)	447 (349–643)	5 (3–9)
SS-A negativity (n = 14)	11,656 (8516–18,319)	521 (301–615)	4 (3–8)
P value	.107	.675	.248
Prednisolone user (n = 21)	10,401 (8516–18,319)	500 (315–643)	4 (3–7)
Non-prednisolone user (n = 13)	10,712 (7058–13,424)	478 (317–625)	4 (3–9)
P value	.535	.986	.468

metabolites in patients with ankylosing spondylitis (AS), serum Trp, Kyn, and 3-hydroxykynurenine levels were found to be remarkably lower in both AS groups compared to the control group, whereas Kyn, quinolinic acid, CRP, erythrocyte sedimentation rate (ESR), and interleukin-6 levels were found to be higher. Conventional treatment and anti-tumor necrosis factor-α treatment were found to be effective in reducing the Kyn/Trp ratio and CRP levels.[44] In our study, there was no correlation between serum Trp, Kyn, and the Kyn/Trp ratio and ESSDAI and ESSPRI disease activity scores in patients with pSS.

Anti-Ro (SS-A) and anti-La (SS-B) autoantibodies were shown to be more frequently positive in patients with polyneuropathy in a study.[45] Trp levels were significantly lower in SSc patients compared to healthy controls according to a recent study.[46] Patients with anti-RNA-polymerase III positivity were shown to have lower Trp levels and higher Kyn levels than patients with anti-centromere and anti-topoisomerase positivity, and autoantibody profile was also found to be considerably correlated with Kyn and Trp levels.[46] Taken together, these findings imply that the higher activity of the KP may be associated with clinical and laboratory manifestations of systemic inflammation.[35,36,45] Although those investigations show a link between neurological or laboratory results and Kyn metabolites, the cause-and-effect link between KP metabolites and these manifestations is yet unknown. Furthermore, the expression of IDO, which metabolizes tryptophan, is known to be upregulated by glucocorticoids.[47] Higher levels of Kyn have also been linked to a decreased percentage of people taking corticosteroids, but not to the frequency of neurological symptoms in pSS patients.[35,36] In our study, although Trp levels were lower in prednisolone users and SS-A positive patients, no statistically significant difference was observed. In addition, no difference was observed between Kyn and Kyn/Trp ratios. This may be related to the small size of the patient population in our cohort.

Trp metabolism through the Kyn pathway has been identified as a central fatigue mechanism.[48,49] In pSS, the interferon-γ-inducible-Kyn pathway could have a role in the neurological symptoms, fatigue, and chronic pain.[45] Since the kynurenine pathway is responsible for the metabolism of around 95% of plasma-free Trp, any change in its activity in response to inflammation is likely to have a disproportionate impact on other Trp or nonkynurenine product pathways. The fact that increased Trp metabolism to kynurenines reduces Trp availability for conversion to 5-HT (serotonin), tryptamine, and melatonin is usually neglected.[50] In a study, a link was made between fibromyalgia and other psychological symptoms such as depression, anxiety, insomnia, psychosis, and neurosis, with fatigue being observed in a group of 106 pSS patients, among whom 32 were considered as fatigued and 74 nonfatigued. However, there was no difference in IDO mRNA levels in pSS patients with and without fatigue in peripheral blood leukocytes.[51] In our cohort, no correlation was found between fibromyalgia and serum levels of Kyn pathway metabolites.

The limitations of our study are mainly due to the limited number of patients. Secondly, almost all of our patients were homogeneous for sicca symptoms and joint involvement, and the number of patients with other extraglandular involvement was very limited. This makes it difficult to establish a relationship between pSS and its involvement and disease activity. Multicenter studies with large patient numbers and different organ involvement are needed to evaluate future usefulness of this pathway in clinical routine.

In conclusion, our data suggest that the Trp metabolic pathway is activated in pSS patients and confirm previous studies that the Kyn pathway is altered. Increased activity of metabolites in the Kyn pathway may have a role in the pathogenesis of pSS. Larger prospective studies are needed to better understand the role of this metabolic route in persons with pSS.

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