Arh. farm. 2026; 76: 122 – 130 *Original scientific paper/Originalni naučni rad*

Sex-specific Associations Between Serum Quinolinic Acid and Disease Severity in Relapsing-remitting Multiple Sclerosis: A Cross-sectional Study

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Received: 05 February 2026; Revised in revised forme: 27 March 2026; Accepted: 05 April 2026

Abstract

The kynurenine pathway (KP) of tryptophan metabolism generates metabolites with both neuroprotective and neurotoxic properties and has been implicated in multiple sclerosis (MS). However, the relationship between KP metabolites, disease severity, and sex remains unclear. Serum levels of quinolinic acid (QA) and kynurenic acid (KA) were analyzed in a cohort of patients with relapsing-remitting MS. Of the 78 initially recruited patients, biomarker data were available for 40 patients, who were included in the final analysis. Disease severity was assessed using the Multiple Sclerosis Severity Score (MSSS) and patients were stratified into mild-to-moderate (MSSS < 6.7) and a severe disease group. Serum QA and KA concentrations were measured by the enzyme-linked immunosorbent assay (ELISA) and analyzed using two-way ANOVA with Sidak post hoc correction. No significant overall sex-related differences in serum QA or KA levels were observed. KA levels did not differ across MSSS categories. Female patients with high disease severity (MSSS > 6.7) exhibited higher serum QA levels compared with those with lower disease severity ($p = 0.032$), although this finding was observed in a very small subgroup of female patients ($n = 3$). These findings suggest a possible sex-related association between elevated serum QA levels and greater disease severity in female patients with RRMS and warrant confirmation in larger studies.

Key words: multiple sclerosis, kynurenine pathway, kynurenic acid, quinolinic acid, sex differences, disease severity

https://doi.org/10.5937/arhfarm76-64396

Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system characterized by inflammation, demyelination, and neuroaxonal damage (1). Although inflammatory activity predominates in the early stages, neurodegenerative mechanisms contribute substantially to long-term disability. Clinical disability is commonly assessed using the Expanded Disability Status Scale (EDSS) (2). However, the Multiple Sclerosis Severity Score (MSSS), which adjusts EDSS for disease duration, provides a more refined estimate of disease severity (3).

The kynurenine pathway (KP) is the principal metabolic route of tryptophan degradation and plays an important role in immune regulation and neuroinflammation (4, 5). Activation of this pathway is induced by proinflammatory cytokines, resulting in the production of several biologically active metabolites.

Among these metabolites, kynurenic acid (KA) is considered neuroprotective due to its antagonistic effects on excitatory amino acid receptors and antioxidant properties, whereas quinolinic acid (QA) is a neurotoxic compound that acts as an agonist of N-methyl-D-aspartate (NMDA) receptors and promotes excitotoxicity, oxidative stress, and mitochondrial dysfunction (6–8). An imbalance favoring QA may therefore contribute to neuronal and oligodendroglial injury.

Alterations in KP metabolism have been reported in MS and other neuroinflammatory disorders (4, 9, 10). Importantly, MS exhibits pronounced sex-related differences in incidence, immune responses, and disease course, and experimental data suggest that sex hormones may influence KP enzyme activity (11). Nevertheless, sex-specific associations between KP metabolites and disease severity in MS remain insufficiently explored. Therefore, the aim of the present study was to investigate serum levels of KA and QA in patients with RRMS, and to examine their relationship with disease severity, with particular emphasis on sex-specific effects.

Materials and methods

Study population

This cross-sectional study included 78 patients diagnosed with relapsing-remitting MS according to the 2017 revised McDonald criteria (12). The patients were recruited from the Neurology Clinic of the Military Medical Academy (MMA), Belgrade, Serbia between March 2018 and July 2019. The cohort consisted of 40 male and 38 female patients. However, complete serum KP metabolites measurements were available for 40 patients due to limited serum volume and sample availability for biochemical analyses, and all biomarker related statistical analyses were therefore restricted to this subgroup. The potential influence of selection bias due to incomplete biomarker availability cannot be excluded. All RRMS patients were receiving disease-modifying therapy (DMT) at the time of blood sampling, including interferon beta preparations, glatiramer acetate, and sphingosine-1-phosphate receptor modulators. Due to relatively small subgroup sizes, further adjustment for specific DMT categories was not feasible. Relapse status at the

time of blood sampling was not systematically recorded and no predefined relapse-free interval was required for study inclusion. The study was approved by the Ethics Committee of the Military Medical Academy (approval No. 4494-1), and all participants provided written informed consent prior to inclusion. Information on menopausal status and hormonal therapy was not available.

Clinical assessment

Based on MSSS values, patients were classified into mild-to-moderate disease (MSSS < 6.7) and severe disease (MSSS > 6.7). Because no universally accepted MSSS threshold exists for defining disease severity categories, patients were stratified using the cohort-specific median MSSS value (6.7).

Biochemical analysis

Venous blood samples were collected under standardized conditions. Serum was separated and stored at –80°C until analysis. The intra- and inter-assay coefficients of variation were <10%. Serum concentrations of QA and KA were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Immusmol SAS, Bordeaux, France; catalog numbers IS-I-0100 and IS-I-0200, respectively). The analytical sensitivity was 6 ng/mL for QA and 0.53 ng/mL for KA. The detection range was 25–2000 ng/mL for QA and 1.40–73.97 ng/mL for KA. All samples were analyzed in duplicate according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro-Wilk test. QA concentrations showed deviation from normal distribution and were logarithmically transformed prior to parametric analyses. Homogeneity of variance was evaluated using Levene's test. Continuous variables are presented as mean ± standard deviation or median (interquartile range), as appropriate. Two-way analysis of variance (ANOVA) was used as the primary analytical approach. The QA/KA ratio was calculated as an exploratory variable and analyzed using the same model. Due to small subgroup sizes and non-normal distribution of the QA/KA ratio, additional non-parametric comparisons were performed using the Mann-Whitney U test. Categorical variables were compared using Fisher's exact test. Correlations between KP metabolites and continuous EDSS and MSSS scores were evaluated using Spearman's rank correlation coefficient. All inferential statistical analyses were conducted in the subgroup of patients with complete biomarker data (n = 40).

Results

Clinical characteristics

Clinical characteristics of the study population are summarized in Table I. Of the total study population (n = 78), complete biomarker data were available for 40 patients,

and subsequent statistical analyses were performed in this subgroup. At the time of blood sampling, all patients were receiving DMT. Within the biomarker subgroup, 25.0% were treated with interferon beta-1b (Betaferon®), 7.5% with interferon beta-1a (Rebif®), 10.0% with glatiramer acetate (Copaxone®), and 57.5% with sphingosine-1-phosphate receptor modulators. Baseline clinical and demographic characteristics did not differ significantly between male and female patients. Due to the relatively small sample size and uneven distribution of patients across treatment subgroups, formal statistical comparisons according to DMT type were not performed, as such analyses would have limited statistical power.

Table I Clinical and demographic characteristics of RRMS patients according to sex

Tabela I Kliničke i demografske karakteristike pacijenata sa RRMS u odnosu na pol

Variable	Males (n = 18)	Females (n = 22)	p value
Age (years)	43.00 ± 9.11	41.64 ± 10.01	0.658
Disease duration (years)	9.22 ± 5.51	7.41 ± 5.35	0.299
BMI (kg/m^2)	25.07 ± 3.06	24.52 ± 2.78	0.561
Smoking status			
Smokers	4 (22.2%)	6 (27.3%)	0.714
Non-smokers	14 (77.8%)	16 (72.7%)	

Values are presented as mean ±SD or number (%). Between-group comparisons were performed using the independent samples t-test or Fisher's exact test, as appropriate.

Serum kynurenine pathway metabolites

No significant differences in serum QA or KA levels were observed between male and female patients when analyzed irrespective of disease severity.

Association with disease severity

A two-way ANOVA performed in the subgroup of 40 patients with complete biomarker data showed no significant main effects of sex ($F = 1.663$, $p = 0.205$) or disease severity ($F = 4.000$, $p = 0.053$) on serum QA levels, nor a significant interaction between these factors ($F = 2.189$, $p = 0.148$). Similarly, a two-way ANOVA showed no significant main effects of sex ($F = 0.034$, $p = 0.855$) or disease severity ($F = 0.914$, $p = 0.346$), nor a significant interaction between these factors ($F = 0.677$, $p = 0.416$) on serum KA levels. However, post hoc analysis revealed significantly higher serum QA levels in female patients with high disease severity compared with those with low disease severity ($p = 0.032$). These findings are illustrated in Figure 1, while detailed subgroup numerical data are provided in Supplementary Table II. Due to non-normal distribution of the QA/KA ratio, and small subgroup sizes, additional non-parametric analyses using the Mann-Whitney U test were performed. Non-parametric analyses yielded consistent

findings, indicating higher serum QA levels in female patients with severe disease compared with those with mild-to-moderate disease. Exploratory analysis of the QA/KA ratio in the subgroup with complete biomarker data revealed no significant main effects of sex ($F = 0.005$, $p = 0.946$) or disease severity ($F = 0.004$, $p = 0.952$), nor a significant interaction between these factors ($F = 0.158$, $p = 0.694$). Corresponding detailed subgroup descriptive statistics (mean ± SD and sample size) are presented in Supplementary Table 2. Spearman correlation analyses performed in the subgroup of patients with complete biomarker data showed no significant associations between serum levels of QA, KA, or the QA/KA ratio and continuous EDSS or MSSS scores (all $p > 0.05$).

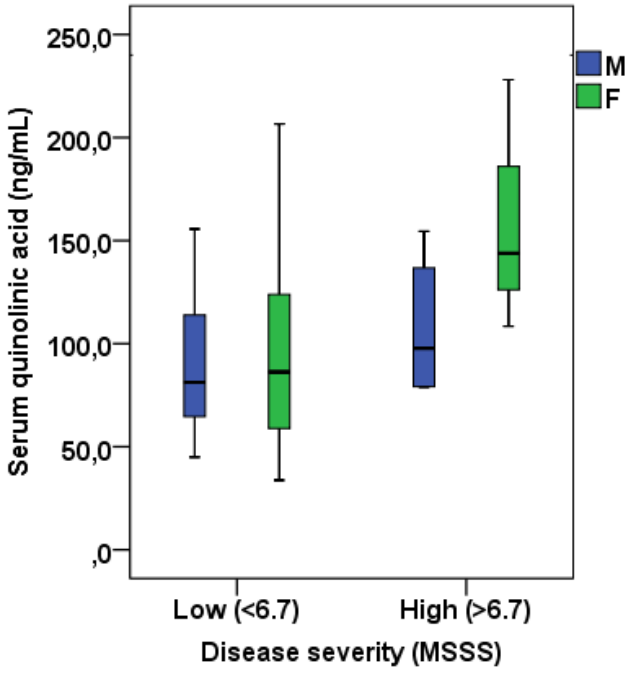

Figure 1. Distribution of serum quinolinic acid levels according to disease severity (MSSS) and sex in the biomarker subgroup (n = 40)

Slika 1. Raspodela serumskih nivoa hinolinske kiseline u odnosu na težinu bolesti (MSSS) i pol u podgrupi biomarkera (n = 40)

Box plots with overlaid individual data points illustrate the distribution values within each subgroup. A statistically significant post hoc difference was observed between females with high MSSS and females with low MSSS ($p = 0.032$, Sidak post hoc following a two-way ANOVA). Statistical analyses were performed on log-transformed quinolinic acid values.

Supplementary

Table II Serum KP metabolite levels according to sex and disease severity

Tabela II Serumski nivoi metabolita kinureninskog puta u odnosu na pol i težinu bolesti

Biomarker (ng/mL)	Males MSSS < 6.7 (n = 12)	Males MSSS > 6.7 (n = 6)	Females MSSS < 6.7 (n = 19)	Females MSSS > 6.7 (n = 3)
QA (ng/mL)	97.58 ± 55.27	107.48 ± 31.95	93.97 ± 44.47	160.12 ± 61.47*
KA (ng/mL)	46.68 ± 26.44	65.71 ± 42.90	53.51 ± 22.17	54.93 ± 9.48
QA/KA ratio	3.07 ± 2.56	2.52 ± 2.03	2.68 ± 3.52	3.08 ± 1.69

$p^* < 0.05$ compared with MSSS < 6.7 within the same sex (Sidak post hoc test). Results in this subgroup should be interpreted with caution due to very small sample size.

Discussion

The principal finding of this cross-sectional study was a possible sex-related subgroup association between elevated serum QA levels and greater disease severity in female patients with RRMS observed in a very small subgroup of female patients. Although the two-way ANOVA did not demonstrate a statistically significant interaction between sex and disease severity on serum QA levels at the model level, analyses within the subgroup of patients with complete biomarker data indicated higher QA concentrations in female patients with severe disease. However, the absence of a statistically significant sex and disease severity interaction in the ANOVA model suggests that this finding should be interpreted cautiously, as it may reflect limited statistical power due to small subgroup sizes rather than a true sex-dependent effect. Importantly, these subgroup findings were additionally confirmed using non-parametric analyses, supporting the consistency of this subgroup observation. The relatively small subgroup size may have limited statistical power. Therefore, this subgroup difference should be considered hypothesis-generating rather than confirmatory. QA is a well-established neurotoxic metabolite of the KP that promotes excitotoxicity, oxidative stress, and cellular injury through NMDA receptor activation (6–8). Experimental studies have demonstrated its detrimental effects on neurons, oligodendrocytes, and blood-brain barrier integrity (6, 13, 14). The observed association between higher QA levels and greater disease severity in female patients is therefore biologically plausible; however, these findings should be interpreted cautiously. The absence of significant changes in KA suggests that disease severity may be more closely related to the increased production of neurotoxic KP metabolites rather than alterations in neuroprotective components. Exploratory analysis of the QA/KA ratio was additionally performed; however, no statistically significant main or interaction effects of sex or disease severity were observed. Additionally, correlation analyses with continuous EDSS and MSSS scores did not reveal significant associations with serum KP metabolite levels, which further

emphasizes the need for cautious interpretation of subgroup findings. Further studies with larger sample sizes are warranted to clarify its potential role in disease severity. The sex-specific nature of this association is noteworthy, as sex hormones and immune mechanisms have been shown to influence KP enzyme activity (11, 15). Several limitations of this study should be acknowledged, including the cross-sectional design, the moderate sample size, and the use of serum measurements that may not fully reflect kynurenine pathway activity within the central nervous system. Additionally, the absence of a statistically significant interaction effect in the ANOVA model may be partly attributable to limited statistical power due to relatively small subgroup sample sizes and substantial biological variability. In particular, the very small number of female patients with high disease severity may also explain the relatively low variability observed for KA levels in this subgroup. Nevertheless, these findings contribute to the understanding of sex-related biological heterogeneity in MS and highlight the potential relevance of KP metabolites in disease severity. From a pharmacological perspective, enzymes of the KP may represent potential targets for modulation of neuroinflammation. Another limitation of the study is the lack of information regarding hormonal status in female patients, including menopausal status and the use of hormonal contraceptives, which may influence KP metabolism. Additionally, relapse status at the time of blood sampling was not systematically recorded. Acute inflammatory activity during relapse may influence KP metabolite levels and could represent a potential confounding factor. The use of cohort-specific median MSSS cut-off to define disease severity may limit comparability with other studies and may reduce statistical power. Furthermore, exploratory correlation analyses with continuous EDSS and MSSS scores did not reveal significant associations with serum KP metabolite levels, further supporting cautious interpretation of subgroup findings.

Conclusion

The present findings suggest a possible sex-related association between elevated serum QA levels and greater disease severity in female patients with RRMS. These findings support further investigation of KP metabolites in MS, particularly through longitudinal and mechanistic studies. Additionally, variability in DMT and relatively small subgroup sizes may have influenced the observed associations.

Declaration of Competing Interest

The author declares no conflicts of interest.

Funding

This study received no financial support.

Author contributions

M.V.: Conceptualization, Data acqusition, Data analysis, Writing- original draft, review & editing.

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Polno-specifične povezanosti između serumskih nivoa hinolinske kiseline i težine bolesti kod relapsno-remitentne multiple skleroze: studija preseka

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Kratak sadržaj

Kinureninski put (KP) metabolizma triptofana proizvodi metabolite sa neuroprotektivnim i neurotoksičnim svojstvima i uključen je u patofiziologiju multiple skleroze (MS). Međutim, odnos između metabolita KP, težine bolesti i polnih razlika još uvek nije u potpunosti razjašnjen. Serumski nivoi hinolinske kiseline (QA) i kinurenske kiseline (KA) analizirani su kod pacijenata sa relapsno-remitentnom MS. Kompletni podaci za biomarkere bili su dostupni kod podgrupe od 40 od ukupno 78 uključenih pacijenata, pa su sve statističke analize sprovedene u toj podgrupi. Težina bolesti procenjena je pomoću skora težine multiple skleroze (Multiple Sclerosis Severity Score-MSSS), a pacijenti su podeljeni u grupe sa blagom do umerenom (MSSS < 6,7) i teškom formom bolesti. Koncentracije QA i KA u serumu određene su metodom enzimskog imunotesta (ELISA), a statistička analiza je sprovedena primenom dvofaktorske analize varijanse (ANOVA) sa Sidak post hoc testom. Nisu utvrđene značajne ukupne razlike u serumskim nivoima QA ili KA između muškaraca i žena. Nivoi KA nisu se razlikovali između MSSS kategorija. Uočene su više koncentracije QA u serumu kod pacijentkinja sa visokom težinom bolesti (MSSS > 6,7) u poređenju sa onima sa nižom težinom bolesti ($p = 0,032$); međutim, ovaj eksploratorni nalaz zasniva se na veoma maloj podgrupi pacijentkinja ($n = 3$). Takva povezanost nije uočena kod muških pacijenata. Ovi rezultati ukazuju na moguću polno povezanu asocijaciju između povišenih serumskih nivoa QA i veće težine bolesti kod pacijentkinja sa RRMS, što zahteva dalju potvrdu u studijama sa većim uzorkom.

Ključne reči: multipla skleroza, kinureninski put, kinureninska kiselina, hinolinska kiselina, polne razlike, težina bolesti