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Persistent Immune–Metabolic Activation in Mucopolysaccharidosis Type IVA Despite Therapeutic Intervention

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Research Article

Keywords: Mucopolysaccharidosis type IVA, Morquio syndrome, Neopterin, Kynurenine pathway, Tryptophan metabolism, Immune–metabolic activation, Enzyme replacement therapy, Hematopoietic stem cell transplantation, Biomarkers, Lysosomal storage disease

Posted Date: June 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9602557/v1

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Additional Declarations: No competing interests reported.

Abstract

Aim

Mucopolysaccharidosis type IVA (MPS IVA) is characterized by lysosomal glycosaminoglycan accumulation and progressive multisystem involvement. Beyond glycosaminoglycan storage, secondary immune and oxidative mechanisms may contribute to disease progression. We aimed to evaluate immune–metabolic activation in MPS IVA by assessing pteridine and kynurenine pathway metabolites alongside conventional inflammatory markers.

Material and Method:

In this cross-sectional study, serum levels of neopterin, biopterin, tryptophan, and kynurenine were measured by high-performance liquid chromatography (HPLC), and IL-1β, IL-6, TNF-α, IFN-γ, chondroitin sulfate, and keratan sulfate were quantified by ELISA in patients with MPS IVA (n = 20) and age- and sex-matched healthy controls. Subgroup analyses were performed among patients receiving enzyme replacement therapy (ERT), those who had undergone hematopoietic stem cell transplantation (HSCT), and untreated patients.

Results

Neopterin levels were significantly elevated in the patient cohort compared with controls ($p < 0.01$), with the highest concentrations observed in the HSCT subgroup ($p = 0.010$) while no difference was detected between the HSCT and ERT groups. Kynurenine concentrations differed significantly among groups (overall $p = 0.010$), with higher levels in the ERT subgroup compared with controls ($p = 0.029$). The kynurenine/tryptophan (KYN/TRP) ratio also showed a significant overall group effect ($p = 0.003$) and was increased in ERT-treated patients compared with controls ($p = 0.002$), without significant differences between treatment subgroups. In contrast, circulating IL-1β, IL-6, TNF-α, and IFN-γ levels did not differ significantly between patients and controls.

Conclusion

MPS IVA is associated with persistent immune–metabolic activation despite long-term therapeutic intervention. The dissociation between normal circulating cytokine levels and elevated neopterin and KYN/TRP ratios suggests ongoing cellular immune signaling beyond conventional inflammatory markers. These findings support the concept that secondary immune–metabolic mechanisms contribute to disease pathophysiology and may influence long-term clinical outcomes in MPS IVA.

INTRODUCTION

Mucopolysaccharidosis type IVA (Morquio syndrome; OMIM #253000) is an autosomal recessive lysosomal storage disorder caused by mutations in the GALNS gene, which encodes N-acetylgalactosamine-6-sulfate sulfatase (GALNS). This results in defective catabolism of chondroitin-6-sulfate and keratan sulfate [1]. The absence of this enzyme impairs the breakdown of chondroitin-6-sulfate and keratan sulfate, leading to the accumulation of glycosaminoglycans (GAGs) in lysosomes in various tissues [1, 2]. Keratan sulfate is essential for the structure and function of proteoglycans in the extracellular matrix of the cornea, cartilage, and bone. In contrast, chondroitin sulfate is distributed throughout cell surfaces and in pericellular and extracellular matrices, where it is covalently linked to a wide range of core proteins [3, 4].

The clinical presentation of MPS IVA is heterogeneous. Classic features include marked short stature, hypoplasia of the odontoid process, kyphoscoliosis, joint laxity, and corneal clouding, while cognitive function is typically preserved [2]. The diagnosis of MPS IVA is established by demonstrating low GALNS enzyme activity in cultured fibroblasts or leukocytes and by confirming homozygous GALNS mutations identified by molecular genetic analysis [3].

Disease-modifying therapies for MPS IVA primarily include enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT). Recombinant human GALNS (elosulfase alfa, Vimizim, Biomarin Pharmaceutical) was approved by the FDA in February 2014 and currently used in the United States, European countries, Turkey, and other developed countries [3]. Enzyme replacement therapy has improved specific biochemical and functional parameters; however, its effect on skeletal pathology and long-term disease progression remains limited. Hematopoietic stem cell-containing bone marrow transplantation theoretically offers the advantage of bone marrow-derived donor macrophages for MPS, providing access to various storage tissues. However, despite improvements in biochemical variables achieved with the newly produced enzyme after HSCT, clinical benefits vary depending disease stage, phenotype, and timing of transplantation, and skeletal dysplasia continues to represent a major therapeutic challenge [3]. Therefore, studies are ongoing on treatments aimed at correcting bone lesions.

Besides lysosomal GAG accumulation, studies indicate that MPS pathogenesis involves more complex pathways, including impaired autophagy, mitochondrial dysfunction, oxidative stress, defective vesicle trafficking and persistent activation of inflammatory pathways. Increased production of reactive oxygen species (ROS) and mitochondrial dysfunction have been observed in several MPS subtypes, indicating a critical role for redox imbalance in disease progression [5].

The inflammatory response is closely linked to antioxidant defense and oxidative stress. Reactive oxygen/nitrogen species (ROS/RNS) can stimulate the release of cytokines and other pro-inflammatory mediators via different signaling pathways. Very little data in the literature show that, in addition to the increase in pro-inflammatory cytokines, MPS IVA patients have impaired enzymatic antioxidant defense, lipid (isoprostanes) and protein (sulfhydryl and di-tyrosine) oxidative damage, and oxidative damage to DNA in leukocytes [6].

Pro-inflammatory and immunoregulatory cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), play central roles in coordinating innate and adaptive immune responses, linking cellular immune activation to systemic inflammatory, metabolic, and tissue remodeling processes [7–11].

Pteridines, especially neopterin and biopterin, are metabolites produced from guanosine triphosphate (GTP) in monocytes, macrophages, and dendritic cells upon stimulation by interferon-gamma (IFN-γ), and are widely used as biomarkers for cellular immune activation and inflammatory burden. Elevated neopterin and altered biopterin levels have been reported across malignant, neurodegenerative, autoimmune, and lysosomal storage disorders, as well as neurodevelopmental conditions, supporting the relevance of the pteridine pathways as indicators of systemic immune–metabolic dysregulation [12–17]

Tryptophan is an essential amino acid that serves as a precursor for protein synthesis and for metabolites of the serotonin and kynurenine pathways. Under conditions of cellular immune activation, tryptophan catabolism is preferentially shunted toward the kynurenine pathway in parallel with neopterin production [18]. The rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO) is transcriptionally induced by interferon-gamma in myeloid, endothelial, epithelial, and dendritic cells, as well as in various tumor cell lines. Enhanced IDO activity results in reduced circulating tryptophan concentrations and a corresponding increase in the kynurenine/tryptophan ratio, a widely used indicator of immune-mediated metabolic regulation and inflammatory tone [19–21].

Despite recent advances in treatment, long-term clinical outcomes of MPS IVA remain unsatisfactory. The contribution of GAG accumulation to oxidative stress, immune activation, and downstream metabolic disturbances, as well as the potential role of antioxidant and immunomodulatory approaches as adjunctive therapies, has therefore received increasing attention.

In this study, we aimed to comprehensively evaluate inflammatory cytokines, pteridine metabolites, and kynurenine pathway intermediates in patients with MPS IVA and to investigate their associations with clinical organ involvement and treatment modality, thereby elucidating their potential roles in disease pathophysiology and as candidate biomarkers of therapeutic response.

METHODS

Ethics committee approval was obtained from İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (13.02.2024–914450) and Scientific Research Unit (08.05.2024/37811).

Patients and Control Subjects

Patients who were followed up after the diagnosis of MPS IVA, participated in this study (20 patients, aged 5–32 years, including 9 males and 11 females, with a mean age of 12.4 years). The average age of patients diagnosed with MPS IVA was 4.5 years. The patients were informed about this study, and

written consent was obtained. Patients diagnosed with MPS IVA were included in the study. All patients had known enzyme levels and genetic mutations.

Out of the 20 patients who participated in our study, four had undergone bone marrow transplantation, 14 were receiving enzyme therapy, and two had never received enzyme therapy before. The average duration of ERT in the patients was 7,1 years (ranging from 2 years 3 months to 17 years 7 months).

Healthy volunteers who come for routine follow-up at Istanbul University Cerrahpaşa Children's outpatient clinic with similar age and sex characteristics, without inflammation, infection, or chronic disease, were included in the control group (aged 6–33, comprising nine males and 11 females, with a mean age of 13.6 years). The control group was informed about the study, and written informed consent was obtained.

For participants under 18 years of age, informed consent was obtained from both the participants and their parents.

Clinical and Laboratory Evaluations of Patients

Outpatient clinic records of the patients participating in the study were reviewed. Each patient's symptoms were examined in detail, and physical examinations were performed. To determine organ involvement, ophthalmology and hearing evaluations, echocardiography, spinal MRI, pulmonary function and walking tests, and DEXA evaluations performed during routine outpatient clinic visits were reviewed. The distribution of findings is shown in Tables 1 and 2.

Table 1
Demographic characteristics of patients with MPS IVA and controls

Age (years), mean ± SD	**Patients (n = 20)**	**Controls (n = 20)**	**p value**
	13.66 ± 7.71	**14.41 ± 7.43**	**0.756**
Male, n (%)	9 (45)	9 (45)	1.000
Female, n (%)	11 (55)	11 (55)	1.000

Table 2. Clinical characteristics of patients with MPS IVA

	Clinical characteristics	n (%)
Cardiovascular Involvement	Tricuspid Insufficiency	3 (15.0)
	Mitral Insufficiency	11 (55.0)
	Aortic Insufficiency	5 (25.0)
Ocular Involvement	Corneal Opacity	15 (75)
Hearing Involvement	Bilateral Sensorineural Hearing Loss	16 (80.0)
	Bilateral Conductive Hearing Loss	4 (20.0)
	Mixed Hearing Loss	5 (25.0)
Skeletal Involvement	Cervical Lordosis	10 (55.6)
	Dorsal Kyphosis	9 (50.0)
	Decreased vertebral body height	9 (50.0)
	Dens Hypoplasia	3 (16.7)
	Beaking of the anterior vertebral body	6 (33.3)
	Spinal canal stenosis	3 (16.7)
	Atlantoaxial subluxation	4 (22.2)
	Kyphoscoliosis	7 (36.8)
	Hip dysplasia	1 (5.6)
Gastrointestinal Involvement	Hepatomegaly	5 (26.3)

Measurements of Pteridine Derivatives, Tryptophan, and Kynurenine

Chemicals and Standards

Chemicals used throughout this study; high-grade purity acetonitrile (ACN), acetic acid (HAc), methanol (MEOH), phosphoric acid, tri(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), formic acid, hydrochloric acid (HCl), trichloroacetic acid (TCA), potassium iodide (KI), iodine (I), creatinine (CRE), sodium hydroxide (NaOH), ammonium formate, sodium acetate (NaAc) (Sigma-Aldrich Química, S.A., Madrid, Spain), KYN and TRP standards were purchased from Sigma (Sigma-Aldrich Química, S.A., Madrid, Spain). Pteridin standards; Pterin-6-carboxylic acid (6PTC), neopterin (NE), pterin (PT), biopterin (BIO), and monapterin (MON) were purchased from Schircks Laboratory (Jona, Switzerland).

Sample Collection and Preparation

Serum samples were collected from the Department of Istanbul University- Cerrahpaşa Medical School, Division of Pediatric Metabolism in Istanbul, Turkey. Blood samples were taken into dry tubes and quickly centrifuged at 3500 rpm for 5 minutes and stored at -80°C until analysis.

Preparation of Serum Samples for Pteridines Analysis

Serum samples were allowed to thaw at room temperature before analysis of the pteridines. For deproteinization 100 µL of 3 M trichloroacetic acid and for oxidation 54 µL of iodine solution (2%/4%, I 2 /I) per 400 µL serum sample was added and vortexed, incubated for 1 hour at room temperature and in the dark. The sample was then centrifuged at 10000 rpm for 10 minutes. It was neutralized with sodium hydroxide (pH: 7) and filtered through a 25 mm 0.2 µm nylon filter [22].

Preparation of Serum Samples for KYN and TRP Analysis

Serum samples were allowed to thaw at room temperature before analysis of KYN and TRP. A 100µL aliquot of serum was added with 200µL of 0.6 M perchloric acid (HClO 4). The mixture was vortexed and centrifuged for 10 minutes at room temperature at 12.000 rpm. The sample was filtered through a 25 mm 0.2 µM nylon filter before injection [22].

Instrumentation and Conditions

Analysis of serum pteridines was performed with an Agilent HP1100 series HPLC (Germany) a scanning fluorescent detector. Data processing was performed with Agilent Open LAB ChemStation software. The wavelength was set to the fluorimetric detection λ ex = 272 nm, λ em = 445 nm [2, 3]. A mobile phase of 2 mM ammonium formate pH 7.1 was used. Chromatography of pteridines was performed by isocratic elution on a Zorbax-SB-Aq C18 (150 mm 4.6 mm, 5.0 m) analytical column. A flow rate of 1.7 mL/min was used in all experiments. The injection cycle volume was 20 µL.

Analysis of serum TRP and KYN concentrations were determined by Nexera-i LC-2040C 3D Plus HPLC (Shimadzu, Kyoto, Japan) equipped with a diode array detector and a scanning fluorescent detector. LabSolutions software was used for PDA, PDA detector, device control, data collection, and data analysis used in the system. Separation of KYN and TRP was achieved by isocratic elution on an AQ-C-18 (3 x 100 mm, 3µm) analytical column at a constant flow rate of 0.5 mL/min at the ambient temperature of 25°C. It consisted of 20 mM sodium acetate (NaAc), 30 mM acetic acid (HAc), and 30 mL/L CH_3OH as the mobile phase. A wavelength of 280 ± 4 nm was chosen for TRP analysis and a wavelength of 234 ± 4nm for KYN. The injection cycle volume was 10 µL.

Method Validation

The method was well-validated as seen in Supplementary Table S1 and S2. The validation of the proposed method was performed according to the Eurachem guidelines 16. Mixed standard solution

was used to create calibration curves. Concentration ranges correspond to the respective levels of the samples. Good linear responses with r > 0.999 were found for all analytes and these data are described in tables. A signal-to-noise ratio of 3:1 was used to determine the LOD and a ratio of 10:1 was used to determine the LOQ. The LOQs of all four analytes were much lower than the lowest standard in the calibration curve.

Serum Pteridines Calibration procedure

Calibration curves were performed with pteridine standard mixtures at six concentration levels as described in Supplementary Table S1.The fluorescence excitation wavelength was 272 nm for all the analytes, and the emission wavelength was 445 nm for the pteridines.

Serum KYN and TRP Calibration procedure

Calibration curves were performed with KYN and TRP levels as described in Table S2. A wavelength of 280 ± 4 nm was chosen for TRP analysis and a wavelength of 234 ± 4nm for KYN.

Calibration curves, limits of detection, limits of quantification, and analytical performance parameters for pteridine and tryptophan–kynurenine analyses are presented in Supplementary Table S1 and S2.

Measurement of Plasma Cytokines and Human Keratan and Chondroitin Sulfate Levels

ELISA Method

In this study, a competitive ELISA method was used for human keratan sulfate, and a sandwich ELISA method for IL-1beta, IL-6, TNF-alpha, and IFN-γ, human chondroitin sulfate following the manufacturer's instructions (BT LAB Bioassay Technology Laboratory, China: E0143Hu QLB00B ELISA kit). IL-1B, IL-6, TNF-alpha, IFN-γ, and chondroitin sulfate present in the samples were added to plates pre-coated with human IL-1B, IL-6, TNF-alpha, IFN-γ, and chondroitin sulfate antibodies, allowing them to bind to the antibodies coated in the wells. Biotinylated human chondroitin sulfate, IL-1 beta, IL-6, TNF-alpha, and IFN-γ antibodies were used.

Streptavidin-HRP is then added, binding primarily to human chondroitin sulfate, IL-1B, IL-6, TNF-alpha, and IFN-γ in the sample, as well as to the biotinylated IL-1B, IL-6, TNF-alpha, IFN-γ, and chondroitin sulfate antibody. After incubation, unbound Streptavidin-HRP is removed during the wash step, substrate solution is added, and color develops depending on the amount of human chondroitin sulfate, IL-6, TNF-alpha, IFN-γ, and IL-1β. The reaction is terminated by adding the stop solution, and the absorbance is measured at 450 nm. To measure plasma keratan sulfate levels, human keratan sulfate is added to a plate previously coated with human keratan sulfate antibody, followed by the addition of biotinylated antigen. The antigens in the sample compete with the biotinylated antigen for binding to the capture antibody. Unbound antigen is removed during a wash step, and avidin-HRP is added. After the unbound antigen is removed during a wash step, the reaction is stopped by the addition of an acidic stop solution,

turning the color yellow, measurable at 450 nm. The concentration of KS in the sample is then determined by comparing the optical density of the samples to a standard curve.

Statistical Analysis

IBM SPSS Statistics 27.0 software was used for statistical analysis in the evaluation of the study findings. Quantitative variables were expressed using mean, standard deviation, median, 25% percentile-75% percentile (Q1-Q3), and min and max values, while qualitative variables were described using descriptive statistical methods such as frequency and percentage. Shapiro-Wilk test and Box Plot plots were used to assess the conformity of the study data to a normal distribution. Student's t-test was used for two-group comparisons of normally distributed variables; One-way ANOVA was used for comparisons of three or more groups, and the Bonferroni test was used to identify the group that caused the difference.

The Mann-Whitney U test was used for two-group comparisons of variables that did not show a normal distribution; the Kruskal-Wallis test was used for comparisons of three or more groups; and the Dunn test was used to identify the group that caused the difference. Spearman's correlation analysis was used to assess the relationships between variables, and the chi-square test was used to compare qualitative data in the study. Results were evaluated at a 95% confidence interval, with significance at a p level of 0.05.

RESULTS

Study Participants

Comprehensive clinical data were systematically collected and analyzed for all participants. Organ involvement was evaluated using standardized ophthalmologic and audiologic examinations, transthoracic echocardiography, spinal magnetic resonance imaging (MRI), pulmonary function testing, six-minute walk tests, and dual-energy X-ray absorptiometry (DEXA). Detailed functional and clinical assessment data are provided in Supplementary Table S3.

Laboratory Assessments

Glycosaminoglycans

Plasma chondroitin sulfate and keratan sulfate concentrations did not differ significantly between patients and controls or across treatment subgroups ($p > 0.05$). Detailed glycosaminoglycan data are presented in Supplementary Table S4.

Plasma Pteridine Profiles

Neopterin concentrations were significantly higher in the patient group compared with healthy controls ($p < 0.01$). Across all MPS IVA subgroups, the highest neopterin levels were observed in patients who had undergone allogeneic hematopoietic stem cell transplantation (HSCT), with a significant overall between-

group effect (p = 0.018). Patients receiving busulfan-based myeloablative conditioning exhibited higher neopterin levels than healthy controls (p = 0.01), while no significant difference was observed between the HSCT and enzyme replacement therapy (ERT) groups (p > 0.05) (Table 3).

Table 3
Pteridine pathway metabolites in patients with MPS IVA and controls

6-Pterin-6-carboxylic acid (nmol/L)	**Patients**	**HSCT**	**Untreated**	**ERT**	**Controls**	**p value**
	5.8 (5.1–6.9)	**6.3 (5–7.7)**	**5.7 (5.3–6.1)**	**5.8 (3.9–22.5)**	**6.1 (5.4–7.9)**	**0.429**
Monapterin (nmol/L)	6.2 (4.3–8.2)	8.8 (1.8–16.8)	10.2 (7.2–13.2)	4.8 (1.1–9.1)	5 (2.3–6.2)	0.223
Isoxanthopterin (nmol/L)	5.3 (3.1–8.3)	8.4 (4–13.2)	5.3 (3.9–6.8)	5.2 (2.7–21.8)	6.4 (3.6–8.7)	0.956
Pterin (nmol/L)	8.9 (5.5–12)	13.4 (9.5–17.5)	6.2 (4.8–7.6)	8.1 (2.9–18.4)	5 (2.9–10.2)	0.077
Neopterin (nmol/L)	10.2 (7.4–17.6)	21.3 (7.6–96)	12.6 (8.1–17)	8.7 (3.6–24.6)	6.3 (2.3–11.2)	0.008
Biopterin (nmol/L)	6.4 (4.8–10.5)	7.8 (3.8–10.8)	3 (2–4.1)	6.7 (3.9–25.9)	5.5 (3–8.9)	0.183
Neopterin / Biopterin	1.2 (1–2.7)	4.1 (1.4–8.9)	4.1 (4.1–4.2)	1.1 (0.8–3)	1 (0.7–1.8)	0.127

Data are presented as median (Q1–Q3). HSCT: hematopoietic stem cell transplantation; ERT: enzyme replacement therapy.

Biopterin levels and the neopterin/biopterin (NE/BIO) ratio did not differ significantly between patients and healthy controls (p = 0.077). Similarly, no significant differences were detected among groups for 6-pterin-6-carboxylic acid (6PTC), monapterin (MON), isoxanthopterin (ISOX), or tryptophan (TRP) (all p > 0.05). Total pterin concentrations differed significantly across groups (overall p = 0.039), driven by higher levels in the HSCT group compared with healthy controls (p = 0.041), with no difference between HSCT and ERT cohorts (p > 0.05) (Table 3).

Kynurenine Pathway Metabolites

Plasma kynurenine (KYN) concentrations were significantly higher in patients than in healthy controls (p < 0.01). The analysis showed a significant difference between groups, with the ERT group showing higher KYN levels than controls (p = 0.029). The kynurenine/tryptophan (KYN/TRP) ratio was markedly

increased in the patient cohort compared to controls ($p < 0.01$) and differed significantly among treatment groups ($p < 0.01$). Pairwise comparisons showed a higher KYN/TRP ratio in the ERT group than in healthy controls ($p < 0.01$), with no significant difference between the HSCT and ERT groups (Table 4).

Table 4
Kynurenine pathway metabolites in patients with MPS IVA and controls

Kynurenine (µmol/L)	**Patients**	**HSCT**	**Untreated**	**ERT**	**Controls**	**p value**
	2.6 (1.7–3.6)	**2.1 (0.5–5.2)**	**1.3 (0.3–2.3)**	**2.7 (1.2–7.3)**	**1.6 (0.9–2.2)**	**0.008**
Tryptophan (µmol/L)	33.4 (27.5–54.9)	28.7 (26.1–81.6)	28.9 (27.8–29.9)	39.3 (20–82.4)	50.9 (41.5–61.3)	0.108
Kynurenine / Tryptophan	7.7 (0.9–23.4)	3.5 (1.7–17.1)	4.7 (0.9–8.4)	8.5 (1.4–23.4)	3.4 (0.4–9.2)	0.002

Data are presented as median (Q1–Q3). HSCT: hematopoietic stem cell transplantation; ERT: enzyme replacement therapy.

Inflammatory Cytokines

Circulating concentrations of TNF-α, IFN-γ, IL-6, and IL-1β did not differ significantly between patients and healthy controls, nor among the HSCT, ERT, and control groups (all $p > 0.05$) (Table 5).

Table 5
Cytokine levels in patients with MPS IVA and controls

TNF–α (ng/L)	**Patients**	**HSCT**	**Untreated**	**ERT**	**Controls**	**p value**
	57.1 (46.8–141.6)	**73 (20.1–380.6)**	**64.8 (47.2–82.4)**	**57.1 (32.2–597.1)**	**46.4 (34.3–1178.4)**	**0.529**
IFN–γ (ng/mL)	59 (39.9–109.3)	65.7 (23–262.8)	72.2 (43.8–100.5)	59 (35.4–267.2)	63.4 (39.3–797.6)	0.314
IL-6 (ng/L)	68.5 (48.7–135.2)	93.6 (31.3–445.1)	78.2 (68.2–88.1)	63.1 (38.6–399.5)	66.9 (44.5–1134)	0.529
IL-1β (pg/mL)	350.8 (240.9–866.1)	251.9 (190.5–1447.4)	476.4 (228.7–724.2)	352.2 (220.9–2819.4)	298.1 (185–14003.9)	0.678

Data are presented as median (Q1–Q3). HSCT: hematopoietic stem cell transplantation; ERT: enzyme replacement therapy.

Association with Clinical Involvement

Correlation analyses revealed a significant negative association between IL-6 levels and the KYN/TRP ratio in patients with corneal haze ($r = -0.618$, $p = 0.014$). No significant correlations were identified between cytokines, pteridine metabolites, or kynurenine pathway intermediates in patients with sensorineural hearing loss or cardiovascular involvement (all $p > 0.05$).

DISCUSSION

Lysosomal storage diseases (LSDs) are a group of more than 70 diseases with lysosomal dysfunction, most of which are inherited as autosomal recessive traits. Due to defects in key enzymatic pathways for macromolecule catabolism, recycling, and signaling, LSDs cause undegraded or partially degraded macromolecules to accumulate in the lysosomes of various tissues, leading to cellular dysfunction. Impairments in phagocytosis, autophagy, exocytosis, receptor recycling and regulation, and intracellular signaling lead to progressive physical symptoms and systemic organ dysfunction [23, 24].

Mucopolysaccharidosis type IV is a lysosomal storage disorder (LSD) characterized by the incomplete degradation of chondroitin sulfate and keratan sulfate resulting from a deficiency of the enzyme N-acetylgalactosamine 6-sulfate sulfatase (GALNS) [1]. Glycosaminoglycans (GAGs) accumulate to a variable degree, causing damage primarily to cartilage, heart valves, and cornea. Lysosomal dysfunction secondary to GAG accumulation contributes to secondary events such as impaired autophagy,

mitochondrial dysfunction, oxidative stress, membrane permeability, and impaired calcium (Ca2+) homeostasis, and ultimately, activation of the NLRP3 inflammasome [25].

In the present study, it is demonstrated that patients with MPS IVA exhibit persistent immune–metabolic activation, as evidenced by increased neopterin levels and elevated kynurenine/tryptophan (KYN/TRP) ratios, despite the absence of significant differences in circulating proinflammatory cytokines. These findings suggest that subclinical cellular immune activation may persist under long-term enzyme replacement therapy (ERT) and following haematopoietic stem cell transplantation (HSCT), even when conventional inflammatory markers appear normal.

Beyond primary lysosomal glycosaminoglycan accumulation, MPS IVA is increasingly recognized as a disorder involving secondary cellular disturbances, including impaired autophagy, mitochondrial dysfunction, oxidative stress, and inflammasome activation. The results of this study support the hypothesis that immune dysregulation constitutes an additional layer of disease pathophysiology, potentially contributing to ongoing tissue damage despite biochemical treatment.

ERT for MPS IV has been used in the United States, Turkey, European countries, and other developed countries since 2014. Studies have shown that elosulfase alfa is well tolerated and provides improvements in endurance, FVC, FEV1, and left ventricular mass index [26, 27].

As demonstrated in previous studies, although ERT has been shown to reduce urinary glycosaminoglycan (GAG) excretion in MPS IVA, systemic and tissue-level responses may remain incomplete. Khan et al. [28] reported that patients' urinary keratan sulfate levels decreased significantly during ERT, while circulating keratan sulfate concentrations remained unchanged compared with untreated patients. These findings suggest that biochemical improvement in excreted substrates does not necessarily reflect normalization at the tissue or systemic level.

Skeletal involvement remains one of the most challenging aspects of MPS IVA management. Tomatsu et al. [29] demonstrated that chondrocytes and extracellular matrix obtained from patients treated with ERT for 6–30 months did not show meaningful reduction in lysosomal vacuolization in bones. Similarly, Yasuda et al. [30] founded no decrease in the need for orthopedic surgery with ERT, and longitudinal growth analyses have not demonstrated significant catch-up growth compared to untreated patients. These studies suggest that, despite biochemical improvements, ERT has limited effect on progressive skeletal pathology, highlighting the need to investigate additional pathogenic mechanisms beyond substrate accumulation [31, 32].

The therapeutic rationale for HSCT in LSDs is based on the engraftment of donor-derived macrophages that can continuously supply the deficient enzyme to affected tissues. Clinical outcomes of HSCT are strongly influenced by patients age, baseline phenotypic severity, donor characteristics, and disease stage at transplantation [33]. Although HSCT has been associated with improvements in various manifestations of MPS, including vision, hearing, cardiopulmonary function, upper airway obstruction, and hepatosplenomegaly. Although its impact on skeletal abnormalities, corneal involvement, and

valvular heart disease remains limited [34–36]. Yabe et al. [37] reported four patients who received allogeneic HSCT, two of these patients' achieved normalization of enzyme activity, with partial improvement in bone manifestations and joint laxity, as well as enhanced respiratory function. However, the results of HSCT in MPS IVA patients appear variable, these findings suggest that HSCT may not fully reverse established skeletal and connective tissue pathologies.

Previous studies have highlighted the role of inflammation and oxidative stress in MPS IVA pathogenesis. Donida et al. [38] showed reduced antioxidant capacity and increased DNA oxidative damage in patients under ERT compared with healthy controls. This study indicate that despite ERT persistent redox imbalance in patients with MPS. In contrast, Delgado et al. [39] reported no significant differences in oxidative/nitrative stress markers or proinflammatory cytokine production in long-term ERT-treated patients. Although IL-10 levels were elevated, suggesting a potential compensatory anti-inflammatory response in MPS IVA patients.

Consistent with previous reports, we did not observe significant differences in circulating IL-1β, TNF-α, IFN-γ, or IL-6 levels between patients and healthy controls [38, 39]. However, serum neopterin concentrations were significantly elevated in the patients with MPS IVA and particularly in those who had undergone HSCT, indicating sustained activation of the cellular immune system. In addition, the kynurenine/tryptophan (KYN/TRP) ratio was significantly increased, especially in patients receiving ERT, supporting ongoing indoleamine 2,3-dioxygenase (IDO)–mediated tryptophan degradation. Because neopterin production and IDO activation are both downstream consequences of IFN-γ–driven macrophage activation, the concomitant elevation of neopterin and KYN/TRP suggests persistent immune–metabolic signaling that may not be reflected by circulating cytokine levels alone. Similar patterns of pteridine and kynurenine pathway activation have been described in other lysosomal storage disorders, including Fabry disease [16], reinforcing the concept that immune–metabolic dysregulation represents a shared secondary mechanism beyond primary substrate accumulation.

Neopterin concentrations were highest in patients who had undergone allogeneic HSCT, even though cytokine profiles did not differ significantly. This observation could be attributable to several underlying mechanisms. First, HSCT initiates a comprehensive immune reconstitution, especially after busulfan-based myeloablative conditioning. This process may lead to sustained activation of donor-derived macrophages and T-cell populations. Because neopterin production marks IFN-γ–mediated macrophage activation, its persistent elevation could indicate ongoing immune remodeling rather than overt systemic inflammation. Second, donor–host immune interactions and low-grade alloimmune activation may contribute to a chronically heightened cellular immune tone even in clinically stable patients. Although none of our patients had overt graft-versus-host disease, subclinical immune activation cannot be excluded. Similar patterns of persistent immune–metabolic activation following HSCT have been reported in other lysosomal storage disorders, suggesting that transplantation does not necessarily normalize downstream inflammatory signaling.Taken together, these findings suggest that HSCT may reduce substrate burden while leaving immune–metabolic pathways partially activated, which may contribute to the incomplete resolution of skeletal and connective tissue manifestations.

IL-6 has been linked with various inflammatory and degenerative ocular conditions, including keratitis, glaucoma, macular edema, and corneal disorders. Experimental evidence suggests that tryptophan metabolites may influence inflammatory pathways in ocular tissues. Matysik-Wozniak et al. [40] showed that changes in tryptophan and kynurenine metabolism affect nitric oxide release and IL-6 production at the ocular surface, suggesting a possible interaction between immune–metabolic pathways and corneal inflammation.

In our cohort, we observed a significant negative correlation between IL-6 levels and the KYN/TRP ratio in patients with corneal clouding. This finding suggests a complex regulatory interplay between local inflammatory signaling and systemic immune-metabolic activation in MPS IVA. Activation of the kynurenine pathway may have context-dependent immunomodulatory effects in corneal tissue, potentially influencing the progression of corneal involvement. Further studies are needed to determine whether modulation of the tryptophan–kynurenine axis may have therapeutic significance for the ocular manifestations of the disease.

Despite long-term ERT, we did not observe significant differences in circulating proinflammatory cytokines between patients and healthy controls. However, the concomitant elevation of neopterin and the KYN/TRP ratio indicates persistent cellular immune activation, supporting a role for T-cell–mediated immune–metabolic pathways in the pathogenesis of MPS IVA.

This study has several limitations. First, due to the rarity of MPS IVA, only a small group of patients was included. The study was designed as cross-sectional; therefore, biomarker assessments were not performed uniformly because the timing of diagnosis and initiation of treatment varied among patients. Although patients did not have known concomitant malignant or inflammatory disorders, comprehensive comorbidity data were limited. The relatively small number of untreated and HSCT patients restricted subgroup comparisons and limits definitive conclusions regarding treatment-specific effects.

In conclusion, immune-metabolic activation persists in patients with MPS IVA despite treatment with ERT or HSCT. The elevated neopterin levels and kynurenine/tryptophan ratios found in the patient group suggest that inflammation and T-cell-mediated immunity contribute to the pathogenesis of MPS IVA. However, no significant difference was found in the levels of conventional proinflammatory cytokines between MPS IVA patients and the healthy control group. Therefore, proinflammatory cytokines are not recommended for monitoring disease severity in MPS IVA patients. Our results support the role of immune activation and inflammation in MPS IVA disease progression and indicate that they may serve as potential targets for further therapeutic approaches. Further longitudinal studies are warranted to determine the clinical significance of pteridine and kynurenine pathway metabolites.

Abbreviations

MPS IVA
Mucopolysaccharidosis type IVA
LSD

Lysosomal storage disease
GAG
Glycosaminoglycan
KS
Keratan sulfate
CS
Chondroitin sulfate
ERT
Enzyme replacement therapy
HSCT
Hematopoietic stem cell transplantation
HPLC
High-performance liquid chromatography
ELISA
Enzyme-linked immunosorbent assay
NE
Neopterin
BIO
Biopterin
KYN
Kynurenine
TRP
Tryptophan
KYN/TRP
Kynurenine-to-tryptophan ratio
IL
Interleukin
TNF-α
Tumor necrosis factor alpha
IFN-γ
Interferon gamma
IDO
Indoleamine 2,3-dioxygenase
DEXA
Dual-energy X-ray absorptiometry
FVC
Forced vital capacity
FEV1
Forced expiratory volume in 1 second
MON

Monapterin
ISOX
Isoxanthopterin
6PTC
6-pterin-6-carboxylic acid
PT
Total pterin.

Declarations

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committees and with the Declaration of Helsinki (1975, revised in 2008). Ethical approval was obtained from the Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Ethics Committee (13.02.2024-914450). Written informed consent was obtained from the parents or legal guardians of all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Istanbul University-Cerrahpaşa Scientific Research Projects Coordination Unit (Project No: TTU-2024-37811).

Authors' contributions

K.B.U., M.S.C., and E.I. contributed to data collection and laboratory analyses. S.K., B.O.H., and T.Z. contributed to patient follow-up and clinical data acquisition. A.Ç.A.Z. contributed to study design and critical revision of the manuscript. E.K. conceptualized and designed the study, performed data interpretation, and drafted the manuscript. All authors critically reviewed the manuscript and approved the final version.

Use of artificial intelligence

Artificial intelligence (AI) tools were used solely for language editing. The authors take full responsibility for the content of the manuscript.

Acknowledgements: This work has been supported by the Scientific Research Projects Coordination Unit of İstanbul University-Cerrahpaşa (project number: TTU-2024-37811)

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest: No conflict of interest was declared by the authors.

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