Serum Neopterin, Biopterin, Tryptophan, and Kynurenine Levels in Patients with Fabry Disease

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Background: Fabry disease is characterized by the accumulation of globotriaosylceramide. Substrate accumulation in lysosomes is thought to trigger an inflammatory response and is responsible for progressive organ damage through the induction of autoimmunity. The levels of pteridine and kynurenine pathway metabolites increase when immune activation is observed and are employed to monitor several diseases and determine prognosis.

Aims: To evaluate we aimed to elucidate the effects of immune activation on the pathophysiology of Fabry disease and to investigate the potential utility of pteridine and kynurenine metabolites.

Study Design: A prospective case-control study.

Methods: In this study, 33 patients with Fabry disease and 33 age- and sex-matched healthy controls were included. Blood pteridine and kynurenine metabolites were studied in both groups. Organ involvement in Fabry disease and its correlation with the pteridine and kynurenine pathways were also investigated.

Results: The patients' neopterin and biopterin levels and the tryptophan/kynurenine ratio were statistically higher than those of the healthy control group ($p < 0.05$). A statistically significant association was found between neopterin levels and hypertrophic cardiomyopathy, cardiac arrhythmias, and GFR values ($p = 0.044$, $p = 0.021$, and $p = 0.030$, respectively), tryptophan and corneal verticillate, hearing loss and tinnitus ($p = 0.010$, $p = 0.009$ and $p = 0.046$, respectively), and kynurenine levels and valvular heart disease ($p = 0.020$).

Conclusion: From the onset of the disease, patients with Fabry disease exhibited elevated levels of inflammation and immune activation. Furthermore, inflammation and immune activation markers can be used as early disease biomarkers.

INTRODUCTION

Fabry disease is an inherited lysosomal storage disease characterized by intracellular globotriaosylceramide (Gb3) accumulation due to a deficiency of the enzyme alpha-galactosidase A (a-GalA). Deficiency of the enzyme a-GalA results in the storage of glycosphingolipids in the lysosomes of various cells, tissues, and organs.[1] The pathophysiological mechanism contributing to the progressive organ dysfunction in the disease involves the gradual accumulation of glycosphingolipids in vascular endothelial cells.[1] The glycosphingolipids accumulated in cells and tissues cause inflammation and fibrosis. Angiokeratomas, corneal and lenticular opacities, acroparesthesia, hypohidrosis, and vascular disease affecting the kidney, brain, and heart are all features of the disease.[1] The definitive diagnosis of the disease involves detecting a-GalA enzyme deficiency and identifying *GLA* gene mutations in both male

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and female patients.[2] The most effective treatment for the disease is enzyme replacement therapy (ERT) administered at regular intervals.[3] ERT improved cardiac and renal functions, decreasing globotriaosylphosphingosine (lyso-Gb3) concentrations in treated patients.[4] Monitoring patients' serum lyso-Gb3 concentrations is considered beneficial for determining treatment efficacy.[5] In addition, new biomarkers are being sought to determine disease severity, organ involvement, and ERT efficacy.

Besides intracellular digestion, lysosomes play a crucial role in antigen processing and presentation in the immune system. Studies examining the effects of lysosomal storage diseases on the immune system suggest that substrate accumulation in lysosomes activates some cascades, leading to an inflammatory response.[6,7] When comparing leukocytes and endothelial samples between patients with Fabry disease and healthy individuals, increased expression of inflammation-associated adhesion molecules was observed in the patients with Fabry disease.[8] Increased inflammation causes oxidative stress and disrupts the structure and function of intracellular proteins. Some proteins with antigenic properties induce autoimmunity, thereby contributing to the progressive organ dysfunction and prognosis of Fabry disease.[9]Pteridines (neopterin, biopterin) are compounds involved in numerous vital biochemical processes in the body, and their concentrations in body fluids are known to increase during infections, inflammation, and immune system activation.[10,11] In some diseases, determining pteridine pathway metabolites or intermediates serves as a crucial biomarker for diagnosis, follow-up, treatment evaluation, and prognosis.[10,12,13] Biopterin, another pteridine compound, is known to vary in concentrations similar to neopterin in several diseases involving immunological stimulation.[14]

Tryptophan, an essential amino acid, is either involved in general protein synthesis in the body or is degraded via the kynurenine pathway. Activated T-cells release the enzyme indoleamine 2,3-dioxygenase (IDO) and induce interferon-γ (*IFN*-γ), which is involved in the rate-limiting step of the reaction in the kynurenine pathway, where tryptophan is degraded to kynurenine. The kynurenine to tryptophan ratio was used to assess IDO activity. The activity of IDO increases during oxidative stress, inflammation, immune activation, and infection.[15] Increased IDO activity has been observed to parallel neopterin levels.[13] In diseases associated with activation of the cellular immune system, increased tryptophan degradation is observed concurrently with neopterin formation. *IFN*-γ stimulates neopterin release and induces IDO, resulting in tryptophan degradation through the kynurenine pathway.[16]

Studies have reported that autoimmune events in patients with Fabry disease are triggered by increased inflammation and oxidative stress.[9,17] In addition, Shen et al.[18] showed that tetrahydrobiopterin deficiency occurs in Fabry disease and may play a role in its pathogenesis. However, a limited number of studies have been conducted on pteridines and kynurenine pathway metabolites in patients with Fabry disease. In this study, we aimed to determine the alterations in the pteridine and kynurenine metabolic pathways in patients with Fabry disease. In addition, we aimed to understand the inflammatory pathogenesis of Fabry disease and evaluate the potential utility of pteridines and metabolites of kynurenine metabolism in improving the disease severity and prognosis.

METHODS

Ethics committee approval was obtained from İstanbul University-Cerrahpaşa, Cerrahpaşa Medical School Ethics Committee (09.07.2021-133730).

Patients and Control Subjects

Patients who were followed up after the diagnosis of Fabry disease in the Division of Pediatric Nutritional and Metabolic Diseases, Department of Pediatrics, İstanbul University-Cerrahpaşa, Cerrahpaşa Medical School participated in this study (33 patients, aged 5-66 years, including 15 males and 16 females, with a mean age of 33.1 years). The average age of patients diagnosed with Fabry disease was 24.1 years (ranging from 2 months to 61 years). The patients were informed about this study, and written consent was obtained. Out of the 33 participants, 27 received ERT. The average duration of ERT in the patients was 7.2 years (ranging from 1 year 7 months to 13 years 8 months).

Healthy volunteers with similar age and sex characteristics, without inflammation, infection, or chronic disease, were included in the control group (aged 6-61, comprising 15 males and 16 females, with a mean age of 33.1 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.

Sample collection and measurement of plasma pteridines levels

Serum neopterin levels have also been shown to follow circadian rhythms, indicating a relationship between melatonin synthesized from tryptophan and tryptophan metabolism.[19,20] To avoid interference from changes in the circadian rhythm, blood samples were collected between 8:00 a.m. and 9:30 a.m. Within 1 h, blood samples were centrifuged at 3,000 rpm for 10 min to separate the serum fraction. The separated serum samples were protected from light and stored at -20 °C without interrupting the cold chain until the day of analysis.

Measurement of plasma pteridine levels

In the study of kynurenine, tryptophan samples were analyzed using a PDA detector in a Nexera-i LC-2040C 3D Plus high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) system. LabSolutions software was used for device control, data collection, and data analysis. The method used in this study was modified based on the work of Zhao[21] Neopterin and biopterin samples were analyzed using an HP1100 model high-performance liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a diode array detector and fluorescence detector (FLD, G1321A). OpenLAB LC ChemStation software was used for HPLC control, data collection, and data analysis. This study was conducted by modifying the study of Kośliński et al.[22]

Clinical and laboratory evaluations of the patients

Outpatient records of patients participating in the study were reviewed. The symptoms of each patient were thoroughly examined, and physical examinations were performed. Organ involvement was determined by analyzing ophthalmological and otological examinations, echocardiography, and 24h rhythm Holter examinations performed during routine outpatient clinic visits. Urinary protein excretion and glomerular filtration rate were calculated over 24 h. Chronic and end-stage renal failure were assessed. The patients' cranial magnetic resonance examinations were evaluated for stroke. The distribution of symptoms and findings is shown in Table 1.

Lyso-Gb3 levels in patients with Fabry

The levels of neopterin, biopterin, tryptophan, kynurenine, and lyso-Gb3 in the blood were concurrently measured in patients with Fabry disease participating in the study.

Statistical analysis

Data analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) for Windows version 22.0 (SPSS Inc, Chicago, IL, USA) and Microsoft Excel (version 2016). Correlations were assessed using Pearson's coefficient and its significance test. The Spearman correlation test was preferred for correlation analysis when the data did not show normal distribution. An independent simple t-test was employed to analyze tests exhibiting a parametric distribution. The Mann-Whitney U test was used in analyzing tests with a nonparametric distribution. A p-value less than 0.05 was considered statistically significant.

RESULTS

Levels of plasma pteridines

The effects of age and sex on the measured parameters were examined, and no difference was found between the two groups ($p > 0.05$). Neopterin levels were higher in the patient group compared to the control group ($p = 0.007$). Similarly, the patient group had higher biopterin levels compared to the control group ($p = 0.048$). Although the patient group had a higher neopterin/biopterin ratio (40%), this difference was not statistically significant ($p > 0.05$). When comparing patients with Fabry disease to the healthy control group, tryptophan levels decreased by 18%, and kynurenine levels increased by 45%. However, there were no statistically significant differences ($p > 0.05$). In patients with Fabry disease, the ratio of kynurenine to tryptophan increased ($p = 0.029$) (Table 2). Six patients remained untreated. No statistically significant differences were found in pteridine and kynurenine pathway metabolites between the patient groups receiving and not receiving ERT ($p > 0.05$).

TABLE 1. Frequency of the Main Symptoms and Signs of Patients.

Symptoms and signs		Frequency	Male	Female
Skin	Facial dysmorphism	27.20%	40%	16.60%
	Angiokeratoma	33.30%	40%	27.70%
Ears	Vertigo	15.10%	40%	11.10%
	Hearing loss	24.20%	20%	11.10%
	Acroparesthesia	96%	73.30%	66.60%
Nervous system	Headache	36.30%	33.30%	38.80%
	Tinnitus	21.20%	26.60%	16.60%
	Hot flashes	36.30%	20%	50%
	Sweating disorder	30%	26.60%	33.30%
	Stroke	9%	0%	16.60%
	Peripheral neuropathy	9%	6.60%	11.10%
Cardiovascular system	Hypertension	18.10%	20%	16.60%
	Hypertrophic cardiomyopathy	30%	46.60%	16.60%
	Valvular heart disease	48.40%	40%	18.10%
	Arrhythmia	12.10%	6.60%	16.60%
Eyes	Cornea verticillata	78.70%	73.30%	83.80%
Gastrointestinal system	Stomach ache	9%	0%	16.60%
	Diarrhea	27.20%	40%	16.60%
	Constipation	27.20%	26.60%	27.70%
	Intestinal gas	24.20%	6%	38.80%
Urinary system	Proteinuria	96%	80%	66.60%
	Decreased GFR	27.20%	26.60%	27.70%

GFR, glomerular filtration rate.

TABLE 2. Levels of Serum Neopterin, Biopterin, Tryptophan, and Kynurenine in the Study Groups.

	Fabry	Control	
n	33	33	
	Median (minimum-maximum)	Median (minimum-maximum)	*p*
Neopterin (nmol/l)	21.56 (0.85-350.64)	5.48 (2.74-8.25)	0.007
Biopterin (nmol/l)	32.63 (1.69-328.85)	13.08 (4.16-205.9)	0.048
Tryptophan (µmol/l)	64.08 (25.3-132.36)	78.88 (29.72-141.95)	0.052
Kynurenine (µmol/l)	2.21 (0.14-14.36)	1.08 (0.11-4.4)	0.130
Kynurenine/tryptophan	39.35 (2.55-317.7)	14.78 (1.27-107.11)	0.029
Neopterin/biopterin	0.47 (0.02-4.34)	0.43 (0.03-1.49)	0.651

Comparison of the levels of pteridine and kynurenine pathway metabolites and lyso-Gb3 in patients

The lyso-Gb3 level surpassed the laboratory's reference value of 1.3 ng/mL in three patients. Despite observing negative correlations between lyso-Gb3 levels and the levels of biopterin (r = -0.250, p = 0.160), tryptophan (r = -0.277, p = 0.119), and kynurenine (r = -0.251, p = 0.159), these correlations were not statistically significant. There was no association between lyso-Gb3 levels and neopterin levels, neopterin/biopterin ratio, or kynurenine/tryptophan ratio. Among three patients with normal lyso-Gb3 levels, one showed no renal or cardiac involvement, whereas the other two had mild cardiac and renal disease. Despite normal lyso-Gb3 levels, there was an increase in pteridine and kynurenine pathway metabolites in all three patients.

Correlation between pteridine and kynurenine pathway metabolite levels and symptoms, clinical findings, and laboratory parameters

A significant association was identified between hypertrophic cardiomyopathy and elevated neopterin levels (p = 0.044). Similarly, a relationship was observed between cardiac arrhythmias and elevated neopterin levels (p = 0.021). Increased kynurenine levels were observed in individuals with valvular heart disease (p = 0.020). A negative correlation was observed between GFR levels, an indicator of renal involvement, and neopterin levels. Increased neopterin levels were found in patients with low GFR levels (p = 0.030). No correlation was identified between proteinuria and metabolites of the pteridine and kynurenine pathways. Tryptophan levels in the patient group were lower than those in the control group. Low tryptophan levels were associated with eye and ear involvement in the disease. Tryptophan levels were related to corneal verticillata (p = 0.010), hearing loss (p = 0.009), and tinnitus (p = 0.046). No statistically significant association was identified between metabolites of the pteridine and kynurenine pathway and skin, nervous system, or gastrointestinal involvement (Table 3).

DISCUSSION

The pathophysiology of Fabry disease involves the accumulation of glycosphingolipids in lysosomes, crucial for various immune system functions, such as signal transduction, autophagy, and antigen presentation. This accumulation is believed to trigger lysosome dysfunction through inflammation and interactions with the immune system.[7,9] Globotriaosylceramide (also known as CD77), a nondegradable glycosphingolipid that accumulates in Fabry disease, functions as a surface differentiation antigen on the cell surface. CD compounds, acting as ligands or receptors on the cell surface, play a role in cell signaling and adhesion. Globotriaosylceramide accumulation is associated with oxidative stress. This leads to uncontrolled inflammation, activating the immune system and potentially worsening the clinical symptoms of the disease.[9]

Pteridines and metabolites of the kynurenine pathway are biomarkers that enhance our understanding of inflammation and the cellular immune system.[11,15] A growing number of studies have demonstrated the clinical importance of these pteridine and kynurenine metabolites, participating in several biochemical processes.[12,13,23] In this study, serum levels of neopterin, a pteridine produced by monocytes and macrophages in response to *IFN-γ* stimulation of the cellular immune system, were examined. Similarly, the neopterin/biopterin ratio, which is known to increase during inflammation and immune activation, was studied. Interferon stimulates the release of neopterin from monocytes and macrophages, along with the enzyme IDO, which degrades tryptophan through the kynurenine pathway.[15] In diseases characterized by activation of the cellular immune system, there is an observed increase in both tryptophan degradation and neopterin formation.[10,13] The precise assessment of the degree of immune activation is facilitated by determining the kynurenine/tryptophan ratio and measuring neopterin concentration, both of which are complementary.[13,16]Serum neopterin and biopterin levels, as well as with the kynurenine/tryptophan ratio, were significantly elevated in patients with Fabry disease participating in this study compared to those of the control group. An elevated kynurenine/tryptophan ratio indicates the activation of the enzyme IDO stimulated by *IFN-γ*. The correlation of this ratio with neopterin, an indicator of immune activation, supports the involvement of inflammatory and immune mediators in the pathogenesis of Fabry disease. Macrophages generate reactive oxygen compounds in addition to neopterin when stimulated by *IFN-γ*. Elevated neopterin levels can serve as an indirect indicator of oxidative stress.[24]

TABLE 3. Clinical Signs of Patients and the Relationship Between Inflammatory Markers.

		Neopterin		Biopterin		Tryptophan		Kynurenine		Neopterin/biopterin		Kynurenine/ tryptophan	
		Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Facial dysmorphism	With	7.43 (8.27)	0.382	20.94 (35.53)	0.707	54.89 (33.7)	0.875	1.21 (1.48)	0.513	0.74 (0.50)	0.580	18.16 (20.60)	0.752
	Without	6.93 (9.33)		22.17 (20.90)		66.01 (34.30)		1.50 (2.26)		0.43 (0.72)		18.87 (36.17)	
Angiokeratoma	With	6.88 (6.23)	0.681	20.94 (23.64)	0.911	73.51 (37.71)	0.941	1.06 (2.26)	0.737	0.46 (0.58)	0.737	17.62 (22.46)	0.881
	Without	8.06 (9.37)		23.15 (23.68)		63.86 (25.88)		1.49 (1.54)		0.49 (0.68)		18.50 (36.61)	
Vertigo	With	7.43 (211.95)	0.109	20.12 (167.87)	0.523	46.55 (34.12)	0.254	0.94 (7.60)	0.750	0.77 (2.43)	0.299	13.96 (178.29)	0.844
	Without	6.81 (9.33)		22.17 (24.04)		69.78 (31.22)		1.43 (1.84)		0.46 (0.59)		18.50 (22.80)	
Hearing loss	With	9.08 (67.37)	0.409	20.53 (22.92)	0.951	45.51 (14.52)	0.009	1.23 (1.08)	0.984	0.75 (0.81)	0.321	25.01 (53.26)	0.183
	Without	6.91 (8.59)		23.41 (23.83)		72.74 (28.25)		1.38 (2.41)		0.46 (0.56)		17.05 (23.95)	
Acroparesthesia	With	6.72 (8.78)	0.201	20.94 (23.49)	0.759	61.15 (36.32)	0.288	1.21 (2.73)	0.745	0.40 (0.63)	0.279	18.84 (39.39)	0.909
	Without	11.41 (9.86)		24.79 (22.05)		73.80 (36.03)		1.43 (1.07)		0.53 (0.68)		17.60 (16.72)	
Headache	With	7.29 (8.06)	0.222	20.29 (22.43)	0.869	66.01 (27.15)	0.158	1.00 (2.26)	0.728	0.44 (0.59)	0.971	14.17 (35.16)	0.222
	Without	6.54 (9.13)		23.41 (25.83)		52.08 (37.17)		1.48 (1.15)		0.47 (0.72)		20.12 (26.19)	
Tinnitus	With	7.16 (83.51)	0.698	20.12 (26.54)	0.949	45.83 (16.36)	0.046	0.39 (1.30)	0.341	0.47 (0.88)	0.651	17.62 (47.52)	0.931
	Without	6.93 (8.57)		24.79 (22.71)		72.18 (29.96)		1.49 (2.19)		0.46 (0.62)		18.50 (26.42)	
Hot flashes	With	6.81 (10.72)	0.608	20.29 (23.11)	0.971	66.01 (31.23)	0.570	1.27 (2.67)	0.942	0.39 (0.59)	0.463	18.90 (36.14)	0.742
	Without	7.43 (8.68)		26.18 (24.58)		63.64 (35.67)		1.38 (1.15)		0.51 (0.73)		18.16 (28.19)	
Sweating disorder	With	7.16 (5.65)	0.864	20.94 (13.64)	0.638	75.99 (30.58)	0.208	1.06 (2.17)	0.593	0.36 (0.26)	0.210	18.16 (26.42)	0.382
	Without	6.91 (9.61)		21.76 (27.33)		62.39 (34.24)		1.49 (1.54)		0.51 (0.71)		18.23 (36.85)	
Stroke	With	15.76 (.)	0.079	20.12 (.)	0.736	53.26 (.)	0.581	1.95 (.)	0.854	0.75 (.)	0.294	35.10 (.)	0.760
	Without	6.89 (8.94)		24.79 (25.24)		66.01 (35.21)		1.355 (1.54)		0.45 (0.64)		17.89 (26.42)	
Peripheral neuropathy	With	15.76 (.)	0.091	19.65 (25.24)	0.480	63.64 (.)	0.760	2.91 (.)	0.713	0.75 (.)	0.324	35.1 (.)	0.807
	Without	6.89 (8.94)		24.79 (25.24)		66.01 (35.91)		1.35 (1.14)		0.46 (0.64)		17.89 (26.42)	
Hypertension	With	8.95 (92.94)	0.269	25.66 (104.95)	0.805	54.07 (36.65)	0.416	1.52 (5.39)	0.339	0.55 (0.59)	0.860	25.63 (126.37)	0.860
	Without	6.91 (9.52)		20.94 (23.64)		67.94 (35.20)		1.38 (1.16)		0.47 (0.72)		18.16 (23.50)	
Hypertrophic cardiomyopathy	With	12.03 (7.23)	0.044	22.17 (17.90)	0.774	71.04 (42.84)	0.465	1.58 (3.74)	0.702	0.62 (0.42)	0.262	17.94 (81.15)	0.848
	Without	6.54 (8.37)		20.94 (26.27)		63.64 (29.44)		1.38 (1.16)		0.40 (0.76)		18.16 (23.50)	

TABLE 3. Continued

		Neopterin		Biopterin		Tryptophan		Kynurenine		Neopterin/biopterin		Kynurenine/tryptophan	
		Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Valvular heart disease	With	12.03 (10.05)	0.065	20.53 (18.85)	0.958	63.86 (45.19)	0.660	1.87 (2.46)	0.020	0.49 (0.56)	0.142	31.76 (52.03)	0.008
	Without	6.72 (4.12)		26.30 (27.10)		67.94 (27.74)		0.94 (1.01)		0.36 (0.64)		14.39 (12.95)	
Arrhythmia	With	6.88 (6.23)	0.021	21.76 (.)	0.589	55.96 (.)	0.706	1.64 (.)	0.957	2.40 (.)	0.386	37.12 (.)	0.589
	Without	8.06 (9.37)		20.94 (26.43)		64.08 (35.20)		1.38 (2.10)		0.47 (0.61)		18.16 (24.25)	
Cornea verticillata	With	6.80 (9.01)	0.341	20.53 (25.24)	0.667	54.07 (31.02)	0.010	1.27 (1.47)	0.966	0.47 (0.61)	0.223	18.23 (37.14)	0.931
	Without	12.06 (8.41)		26.30 (21.56)		81.75 (33.71)		1.92 (2.41)		0.40 (1.15)		18.16 (23.50)	
Stomach ache	With	7.16 (.)	0.854	19.65 (.)	0.951	72.74 (.)	0.854	3.48 (.)	0.540	0.36 (.)	0.324	47.84 (.)	0.760
	Without	6.93 (9.61)		22.17 (24.49)		63.86 (36.37)		1.35 (1.14)		0.49 (0.73)		17.89 (22.35)	
Diarrhea	With	7.16 (6.06)	0.664	11.73 (12.87)	0.014	54.89 (23.85)	0.526	0.99 (3.17)	0.416	0.82 (0.79)	0.048	18.16 (36.42)	0.635
	Without	6.89 (10.88)		28.34 (20.74)		72.18 (36.24)		1.49 (1.09)		0.43 (0.50)		18.23 (31.02)	
Constipation	With	6.91 (7.99)	0.428	20.94 (19.85)	0.635	63.64 (33.83)	0.580	1.98 (2.89)	0.476	0.36 (0.94)	0.635	24.22 (35.07)	0.752
	Without	8.19 (8.46)		24.79 (24.35)		66.01 (31.97)		1.35 (1.09)		0.47 (0.61)		17.33 (39.86)	
Intestinal gas	With	7.03 (6.50)	0.870	33.78 (20.41)	0.102	70.34 (19.38)	0.263	1.00 (2.71)	0.870	0.25 (0.18)	0.060	13.89 (38.34)	0.565
	Without	6.95 (10.70)		20.94 (23.30)		24.89 (37.54)		1.48 (1.19)		0.51 (0.56)		18.84 (21.76)	
Proteinuria	With	7.16 (9.97)	0.110	20.94 (21.62)	0.296	67.94 (36.36)	0.765	1.21 (1.14)	0.586	0.40 (0.54)	0.744	17.62 (21.71)	0.758
	Without	6.74 (8.51)		18.73 (26.10)		58.08 (38.30)		1.65 (3.21)		0.74 (0.66)		26.32 (74.20)	
Decreased GFR	With	10.4 (25.55)	0.030	28.28 (13.73)	0.168	58.72 (45.62)	0.441	1.67 (1.24)	0.938	0.53 (0.52)	0.591	20.22 (47.11)	0.722
	Without	6.54 (5.16)		14.17 (26.27)		64.08 (25.93)		1.33 (2.82)		0.46 (0.74)		17.62 (26.90)	

GFR, glomerular filtration rate.

Three patients had normal lyso-Gb3 levels and presented with the classic form of the disease. Interestingly, among these, one patient with the classic form, who had not yet started ERT and showed no signs of affected organs, exhibited elevated levels of pteridine and kynurenine metabolites despite lyso-Gb3 levels being within the normal range. The other two patients whose lyso-Gb3 levels were within the normal range received ERT. These two patients showed multisystemic disease involvement, with elevated metabolites of the pteridine and kynurenine pathways found despite normal lyso-Gb3 levels. Similarly, another patient with the classical form of the disease, not receiving ERT but showing mild symptoms of renal and cardiac disease, displayed elevated levels of pteridine and kynurenine metabolites, mirroring the concurrently elevated lyso-Gb3 levels.

Lyso-Gb3 levels were not related to the biomarkers studied. However, patients had high levels of pteridine and metabolites of the kynurenine pathway before the onset of organ involvement and lyso-Gb3 accumulation. Similarly, high levels of pteridine and kynurenine metabolites were found in individuals whose lyso-Gb3 levels were within the normal range but who had mild symptoms of renal and cardiac disease. Therefore, it is suggested that pteridine and kynurenine metabolites may serve as early biomarkers of inflammation in Fabry disease, indicating the onset

and progression of organ dysfunction. These biomarkers appear to be positive earlier in the disease course, before the onset of lyso-Gb3 accumulation.

To date, lyso-Gb3, along with a-GalA activity in males, is the only biomarker used for diagnosing Fabry disease. Histopathological analysis can be used to confirm the diagnosis of Fabry disease in difficult cases; however, it is invasive and may not always be accessible. The results of this study suggest that specific markers of inflammation and immune activation may be even more sensitive than Lyso-Gb3 in the early stages of the disease. Thus, these markers appear to be potential tools for the diagnosis and treatment of this disease.

Renal function gradually deteriorates over time in untreated patients. Although Gb3 accumulates in all renal cells, no direct association between Gb3 levels and renal disease has been demonstrated.[25] In our study, no relationship was found between the patients' lyso-Gb3 and GFR levels. However, a statistically significant increase in neopterin levels with decreasing GFR was observed ($p = 0.030$). Neopterin levels in parallel with the glomerular filtration rate help to elucidate the role of inflammation and immune activation in the development and progression of renal disease.

In their study on neopterin levels in patients with chronic heart failure, Caruso et al.[23] found a positive correlation between left ventricular enlargement and neopterin levels. Therefore, it was suggested that the activation of the monocyte and macrophage systems plays an important role in developing cardiac dysfunction. In this study, the cardiovascular system was affected in 88.4% of patients. The most common finding was valvular heart disease, followed by hypertrophic cardiomyopathy, hypertension, and arrhythmia. Kynurenine levels were higher in patients with valvular heart disease ($p = 0.020$). Neopterin levels were higher in patients with hypertrophic cardiomyopathy and cardiac arrhythmias ($p = 0.044$ and $p = 0.021$, respectively). Considering these results, studying the pteridine and kynurenine signaling pathways would enhance our understanding of cardiovascular involvement, progression, and prognosis in Fabry disease.

When analyzing the symptoms and signs of the patients, a statistically significant correlation was found between hearing loss and tinnitus, both associated with reduced tryptophan levels ($p = 0.009$ and $p = 0.046$, respectively). In recent years, several studies have suggested that hearing loss and tinnitus may be related to neuroinflammation. In a study by Haider et al.,[26] examining the role of oxidative stress and inflammation in patients with tinnitus and age-related hearing loss, reduced interleukin (IL)-10 levels were observed in patients with tinnitus. Shulman et al.[27] found that inflammation and tumor nectosis factor (TNF)-α contribute to the etiology of tinnitus, and blocking TNF-α has a therapeutic effect on tinnitus. The results of this study further support the role of inflammation in the etiology of otological involvement in Fabry disease. Suppressing inflammation in patients with Fabry disease opens new avenues for preventing and treating otological involvement in the disease.

Cornea verticillata, resulting from glycosphingolipids deposition, serves as a direct indicator of lysosomal accumulation, offering insights into the severity of Fabry disease.[28] In this study, Fabry patients with cornea verticillate showed a decrease in tryptophan levels ($p = 0.010$). Based on this finding, monitoring tryptophan levels could help determine the severity of the disease and track its progression.

In this study, no significant difference was found in the metabolism of pteridine and kynurenine metabolites between the patient groups with and without ERT. Several reasons may account for this. The number of patients not receiving ERT was relatively small, and the differing disease stages among those receiving ERT might affect pteridine and kynurenine metabolites. The impact of ERT treatment on these metabolites is unknown, limiting the discussion on the role of pteridine and kynurenine metabolic pathways in ERT efficacy. Therefore, studies involving large groups of patients before and during ERT are needed.

Fabry disease can be effectively treated with ERT. In addition to ERT and chaperone therapy, recent developments in gene therapy also hold promise for improving disease prognosis.[29] Understanding the contribution of inflammation and immune activation to the overall pathophysiology of FD will be an important factor in developing new and effective therapies. By slowing disease progression, ERT may prevent permanent loss of organ function. Therefore, early initiation of ERT is considered critical to significantly alter the disease's natural history.[30]

This study has several limitations. The individuals included in the study were at different disease stages. Hence, the timing of marker analysis varied from patient to patient. Patients did not have known cancer, immune, or inflammatory diseases as concomitant diseases. However, complete data on all potential concomitant diseases were unavailable. Most patients were receiving ERT, limiting the discussion on the role of the pteridine and kynurenine pathways in the initial diagnosis of disease and the efficacy of ERT.[5] As most patients in this study received ERT, lyso-Gb3 levels were suppressed. This may explain why no correlation was observed between lyso-Gb3 and the products of the pteridine and kynurenine pathways.

In conclusion, increased inflammation and immune activation have been observed in patients with Fabry disease, both with or without treatment, from the onset of the disease. In addition to ERT, targeted therapies for inflammation and immune activation may be beneficial in managing the disease. Furthermore, markers of inflammation and immune activation can serve as early biomarkers for the disease in previously untreated patients, aiding in the interpretation of mutations associated with Fabry disease and informing decisions on initiating ERT.

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