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Early prediction of retinopathy of prematurity using targeted metabolomic profiling of 6-hydroxymethylpterin in preterm infants

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Abstract

Retinopathy of prematurity (ROP) is a preventable cause of childhood blindness in extremely preterm infants, yet early biomarkers are lacking. In this pilot study, we utilized targeted metabolomic profiling of blood samples to measure plasma 6-hydroxymethylpterin (6PTC) levels in 18 preterm neonates. Results showed significantly higher 6PTC levels in infants who developed ROP compared to those who did not ($p=0.035$), with receiver-operating characteristic analysis yielding an AUC of 0.831. These findings suggest that 6PTC may serve as a non-invasive early biomarker reflecting oxidative stress in ROP. However, the modest sample size warrants further validation in larger, longitudinal cohorts. Given the limited sample size, these results should be interpreted within the context of a pilot, hypothesis-generating study.

Keywords 6-hydroxymethylpterin, Targeted metabolomics, Neonatal biomarker, Retinopathy of prematurity, Oxidative stress

Introduction

Retinopathy of prematurity (ROP) is a vasoproliferative retinal disorder that primarily affects preterm infants and is a leading cause of preventable childhood blindness worldwide. The condition results from disrupted retinal vascular development, exacerbated by postnatal environmental stressors such as fluctuating oxygen levels and oxidative injury [1, 2]. While advances in neonatal intensive care have improved survival among extremely preterm infants, they have also contributed to an increased population of infants at risk for ROP.

The pathogenesis of ROP is multifactorial and strongly influenced by oxidative stress and inflammation. Preterm neonates are particularly susceptible to oxidative injury due to immature antioxidant defense systems and enhanced exposure to pro-oxidant stimuli such as supplemental oxygen, infections, and parenteral nutrition [3,

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4]. Oxidative stress not only contributes to direct retinal endothelial damage but also promotes angiogenic signaling, further exacerbating pathological neovascularization [5].

Metabolomics is the comprehensive study of small-molecule metabolites in biological systems, aiming to capture metabolic alterations associated with physiological or pathological processes [6, 7]. Recent studies in this field have highlighted the potential of such small-molecule biomarkers to identify infants at risk for ROP prior to clinical manifestation [8, 9]. Among these, pteridine derivatives—particularly 6-hydroxymethylpterin (6PTC), a byproduct of folate metabolism and tetrahydrobiopterin (BH4) degradation—have garnered interest due to their association with nitric oxide synthesis and ROS buffering. In a detailed metabolomic study, Monni et al. [10] reported distinct alterations in pterin derivatives among preterm infants, suggesting their role in neonatal oxidative physiology. Supporting this, Yang et al. [5] have demonstrated key metabolic changes in plasma samples, highlighting the potential of metabolic profiling in identifying non-invasive early markers of disease progression. Despite these advances, reliable biomarkers for predicting ROP development remain elusive. Such biomarkers would be especially valuable in resource-limited settings and warrant further investigation through prospective clinical studies.

6-hydroxymethylpterin (6PTC) has been shown to accumulate under conditions of oxidative stress and immune activation [11]. However, its role in neonatal diseases, and specifically in ROP, has not yet been systematically evaluated. This study was designed as an exploratory pilot investigation aimed at generating preliminary data on oxidative biomarkers and aims to assess early postnatal pteridine levels and explore their association with ROP development in preterm infants.

Materials and methods

Study design and participants

This prospective observational pilot study was conducted between December 2024 and June 2025 in the neonatal intensive care unit (NICU) of the Cerrahpaşa Faculty of Medicine, Istanbul University-Cerrahpaşa. Inclusion criteria were based on the 2021 Turkish Neonatology Society Retinopathy of Prematurity (ROP) screening guidelines, which recommend ROP screening in infants born at < 34 gestational weeks and/or with a birth weight < 1700 g [12]. To exclude the contribution of sepsis to oxidative stress, samples from infants with elevated C-reactive protein levels were not included in the study.

A total of 18 preterm infants who met the screening criteria and were hospitalized in the NICU during the study period were included. As this was a pilot study, the number of patients was not calculated beforehand; the number of participants was determined by the availability of suitable residual serum samples.

Sample collection and storage

Residual serum samples were obtained from routine blood samples collected on postnatal day 14, which coincided with the standard biochemistry work-up schedule. After clinical use, the remaining serum was aliquoted and stored at − 80 °C until analysis. No additional blood was drawn for research purposes.

Laboratory analysis

Quantification of pteridine derivatives (including neopterin, biopterin, isoxanthopterin, monapterin, and 6-hydroxymethylpterin) was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The protocol followed the same method described in the research conducted by Ozer et al. [11], with slight modifications adapted to neonatal serum matrix. Prior to the analysis of pteridine compounds, serum samples were thawed at room temperature. To each 400 µL serum aliquot, 100 µL of 3 M trichloroacetic acid and 54 µL of iodine solution (2% I_2/4% KI) were added and thoroughly mixed using a vortex. The resulting mixture was incubated in the dark at room temperature for one hour. Subsequently, the samples were centrifuged at 10,000 rpm for 10 min. The supernatant was then neutralized with sodium hydroxide and passed through a 0.2 µm nylon filter (25 mm diameter) before further analysis. Serum pteridine derivatives were expressed as nmol/L.

All samples were prepared and analyzed in the Pediatric Metabolic Diseases Laboratory of Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, blinded to ROP outcome.

Outcome classification

Infants were categorized into two groups based on ROP status:

- - ROP (+): Infants who developed any stage of retinopathy of prematurity.
- - ROP (–): Infants who did not develop ROP during the routine screening follow-up.

ROP diagnosis and classification were performed by a single experienced pediatric ophthalmologist specialized in ROP screening, in accordance with the International Classification of ROP guidelines [13].

Statistical analysis

As this was an exploratory pilot study primarily aimed at generating preliminary data, no formal power analysis was conducted. Statistical analyses were performed using

Table 1 Clinical characteristics of the study groups ($n = 18$). Values are presented as median (IQR); Mann–Whitney U test was used

Variable	Non-ROP Group ($n = 13$)	ROP Group ($n = 5$)	*p*-value
n (%)	13 (72.2%)	5 (27.8%)	–
Birth Weight (g)	1540 [1100–1640]	1360 [1195–1530]	0.8435
Gestational Age (weeks)	32.0 [29.0–33.0]	30.1 [28.6–31.0]	0.3733

Table 2 Pteridine derivative concentrations in the ROP and non-ROP groups. All values are presented as median (IQR); Mann–Whitney U test was used for comparisons

Marker	Non-ROP Group	ROP Group	*p*-value
6PTC (nmol/L)	19.59 [17.39–25.41]	26.58 [23.87–36.52]	0.0350
Biopterin (BIO) (nmol/L)	22.08 [14.10–26.05]	48.33 [22.75–68.72]	0.2075
NEO/BIO Ratio	5.75 [3.37–8.39]	2.34 [2.03–5.81]	0.5028
Isoxanthopterin (ISOX) (nmol/L)	36.83 [26.53–54.00]	57.07 [37.76–403.32]	0.3359
Neopterin (NE) (nmol/L)	132.42 [49.17–190.13]	98.15 [93.16–132.07]	0.7750
Monapterin (MON) (nmol/L)	9.23 [8.02–13.18]	13.69 [11.69–29.47]	0.0754
Pterin (PT) (nmol/L)	30.78 [26.14–53.72]	31.66 [26.11–125.64]	0.7750

SPSS version 22. Group comparisons were conducted using the Mann–Whitney U test for non-parametric variables. ROC (receiver operating characteristic) curve analysis was used to assess the predictive ability of each pteridine marker. Cut-off values were determined using the Youden Index.

Ethics and funding

This study was approved by the Clinical Research Ethics Committee of Istanbul University-Cerrahpasa (Approval No: 1150502, Date: November 19, 2024). In accordance with national and institutional regulations, approval from this ethics committee requires adherence to the ethical principles outlined in the Declaration of Helsinki. Therefore, the study was conducted in full compliance with the Helsinki Declaration. As the study utilized anonymized residual serum samples obtained from routine clinical care and involved no additional procedures, the requirement for informed consent from parents or legal guardians was formally waived by the ethics committee.

This study was, and the recently commenced expanded cohort study is being funded by the Scientific Research Projects Coordination Unit (BAP) of Istanbul University-Cerrahpaşa under project code TSA-2025-38429.

Results

A total of 18 preterm infants who met the inclusion criteria were included in this pilot study. Based on the ophthalmologic examination results, 5 infants (27.8%) developed retinopathy of prematurity (ROP group), while 13 infants (72.2%) did not (non-ROP group).

Baseline clinical characteristics

The clinical characteristics of the two groups are summarized in Table 1. There were no statistically significant differences in birth weight ($p = 0.8435$) or gestational age ($p = 0.3733$) between the groups. Median birth weight in the ROP group was 1360 g (IQR: 335) compared to 1540 g (IQR: 540) in the non-ROP group. Similarly, median gestational age was 30.1 weeks (IQR: 2.4) in the ROP group versus 32.0 weeks (IQR: 4.0) in the non-ROP group.

Pteridine derivative concentrations

The measured levels of seven pteridine-related compounds, including the NEO/BIO ratio, are presented in Table 2. Among all markers evaluated, only 6PTC showed a statistically significant difference between groups, with higher median levels in infants who developed ROP ($p = 0.035$). No other marker reached statistical significance ($p > 0.05$ for all comparisons).

ROC curve analysis for 6PTC

As only 6PTC levels demonstrated a statistically significant difference between groups, ROC analysis was performed solely for this marker (Fig. 1). The area under the ROC curve (AUC) was 0.831, indicating good discriminative performance in predicting ROP development. The 95% confidence interval (CI) for the AUC was 0.640–1.000, with a p-value of 0.034. The optimal cut-off value, determined using Youden's Index, was 22.45, providing 100% sensitivity and 69% specificity.

Discussion and conclusion

Discussion

In this pilot study, we investigated the potential role of various pteridine derivatives as predictive biomarkers for the development of ROP in preterm infants. Among the seven metabolites analyzed, only 6PTC levels were found to be significantly elevated in infants who developed ROP compared to those who did not. ROC curve analysis further supported its clinical relevance, yielding an AUC of 0.831, with an optimal cut-off value of 22.45, demonstrating 100% sensitivity and 69% specificity. Conversely, other pteridine-related markers such as neopterin, biopterin,

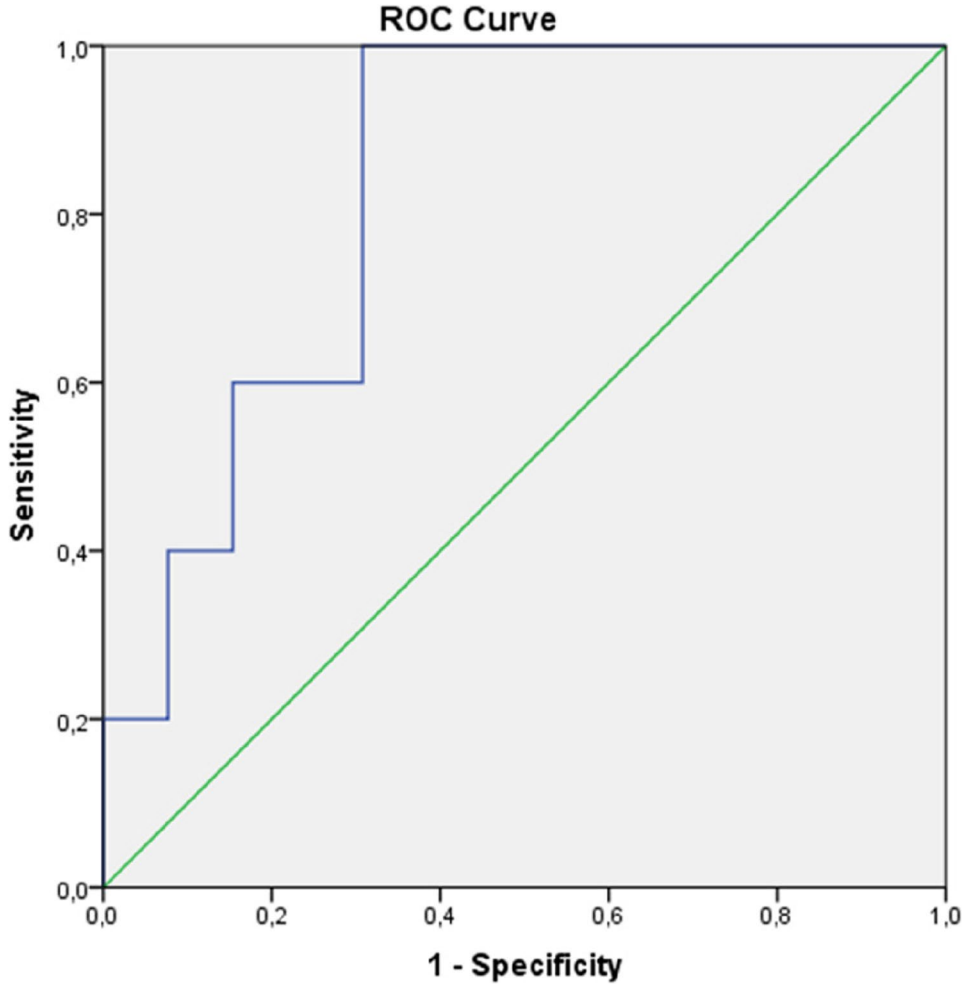

Fig. 1 ROC curve for 6PTC in predicting ROP (AUC:0.831)

monapterin, isoxanthopterin, total pterin, and the NEO/BIO ratio did not exhibit statistically significant differences between groups. Additionally, no significant differences were observed in gestational age or birth weight between the ROP and non-ROP groups, indicating that metabolic profiles may provide supplementary predictive value beyond traditional clinical risk factors.

The identification of 6PTC as a potential biomarker for ROP in this study is consistent with growing evidence that links pteridine derivatives to oxidative stress. 6PTC, a byproduct of folate metabolism and tetrahydrobiopterin (BH4) degradation, has been shown to accumulate under conditions of reactive oxygen species (ROS) exposure and immune activation [14]. The retina, due to its high oxygen consumption and lipid-rich structure, is especially vulnerable to oxidative injury—a key driver in ROP pathogenesis [15, 16]. Notably, animal models and preterm neonate studies have confirmed that fluctuating oxygen levels during postnatal adaptation can induce redox-sensitive signaling cascades and endothelial dysfunction in retinal vessels [17, 18]. Our findings, showing significantly higher 6PTC levels in infants with ROP and a strong diagnostic performance, suggest that 6PTC may reflect localized oxidative processes specific to the developing retina and could serve as a surrogate for early vascular stress in susceptible neonates. However, 6PTC is a systemic biomarker and does not specifically reflect retinal tissue conditions. Its elevation may reflect a broader redox imbalance rather than retinal-localized pathology [11].

Although other pteridine derivatives were measured, including neopterin, monapterin, isoxanthopterin, and the neopterin/biopterin ratio, none displayed statistically significant differences between groups. This may reflect both technical and biological factors. From a pathophysiological standpoint, these markers may be more representative of systemic inflammatory responses than localized retinal stress. Neopterin, for example, is a well-established indicator of macrophage activation but is less tissue-specific [19]. Moreover, large-scale metabolomic analyses of ROP plasma samples have consistently shown that perturbations in amino acid metabolism, fatty acid

oxidation, and mitochondrial energetics—not isolated pteridines—are the primary discriminators of disease [5]. It is also worth noting that due to the small cohort size, modest biomarker changes may have gone undetected. However, the lack of significant alterations in multiple other metabolites across several studies strengthens the hypothesis that 6PTC may offer a more targeted oxidative biomarker profile in ROP than other pteridines.

Future studies should aim to validate 6PTC's diagnostic utility in larger, multicenter cohorts using longitudinal sampling and multi-omics integration, as suggested in recent comprehensive reviews exploring metabolomic, cytokine, and oxidative stress-based biomarkers for ROP [20]. Additionally, the recent animal model data by Yuhang et al. [18] highlight metabolic interplay between the retina and systemic compartments under hyperoxic conditions. Combined with genomics, lipidomics, and clinical indicators, pteridine profiling — particularly 6PTC — could contribute to a robust, non-invasive panel for early ROP risk prediction [16, 18].

The primary limitation of this study is its small sample size, which limits the statistical power and external validity of the findings. As a pilot investigation, no prior sample size calculation was performed, and the study was not powered to detect subtle metabolic differences. Again, due to the small number of ROP cases, we were unable to conduct a stratified analysis by disease stage or sex. Additionally, biomarker profiling was limited to a single time point; the temporal evolution of pteridine metabolites across ROP stages remains unknown. Methodological limitations such as analytical variability in HPLC sensitivity and storage-induced degradation may also have influenced some measurements. Importantly, we did not integrate these biomarkers with clinical or imaging-based ROP staging, which could improve predictive modeling.

Additional limitations include the single-center design, lack of external validation, and the inability to perform stratified analysis by sex or ROP stage due to limited case numbers.

Conclusion

In conclusion, our pilot data suggest that 6PTC may serve as a promising early biomarker for the development of retinopathy of prematurity in preterm infants. Its association with oxidative stress and favorable diagnostic performance highlights its potential utility in neonatal risk stratification. However, the clinical implementation of 6PTC as a predictive tool requires further validation in larger, prospective studies that incorporate longitudinal sampling and correlate biochemical findings with clinical outcomes and retinal imaging. Our research group is currently conducting an expanded cohort study to validate and refine these preliminary findings, aiming to clarify the role of pteridine metabolism in ROP pathogenesis and its integration into future screening algorithms.

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Authors' contributions
Conceptualization: ZAÜ, BBO, HD, AD, EU Methodology: ZAÜ, MŞC, AÇAZ, ZMV Investigation: ZAÜ, HD, BBO, LA, AD, EU Formal analysis: ZAÜ, HD, LA, AD, EU Resources: ZAÜ, HD, LA Data curation: ZAÜ, US, MŞC Writing – Original Draft: ZAÜ, HD Writing – Review & Editing: AÇAZ, ZMV Supervision: AÇAZ, ZMV Revision: ZAÜ.

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

Declarations

Ethics approval and consent to participate
This study was approved by the Clinical Research Ethics Committee of Istanbul University-Cerrahpasa (Approval No: 1150502, Date: November 19, 2024). Informed consent was not required, as only residual serum samples from routine clinical care were used and all data were anonymized.
As the study utilized anonymized residual serum samples obtained from routine clinical care and involved no additional procedures, the requirement for informed consent from parents or legal guardians was formally waived by the ethics committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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