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Circadian rhythms and asthma: exploring the impact of circadian clock proteins on childhood asthma management

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Abstract
Background: Asthma is a chronic inflammatory disorder characterized by airway inflammation and hyperresponsiveness, significantly impacting children's quality of life. Despite optimal treatment, some children experience poor asthma control, partly attributed to circadian rhythm disturbances.
Objectives: This study aimed to evaluate circadian clock protein levels and their relationship with asthma control and sleep quality in children.
Methods: Patients with asthma aged 8–17 years and age-matched healthy controls were enrolled. Pulmonary function was assessed using spirometry, asthma control via the Asthma Control Test (ACT), and sleep quality using the Children's Sleep Habits Questionnaire (CSHQ). Serum circadian protein levels (BMAL1, CLOCK, CRY1, CRY2, PER1, and PER2) were quantified and compared between groups.
Results: Asthmatic children had significantly elevated levels of BMAL1, CLOCK, CRY1, PER1, and PER2 compared to controls ($p<0.01$); however, CRY2 was not significantly different. Poor sleep quality was associated with higher levels of BMAL1, PER1, and PER2 ($p<0.01$). Elevated circadian protein levels correlated with poorer asthma control and reduced pulmonary function (FEV1, FEV1/FVC, PEF; $p<0.05$). Individually, PER2 and BMAL1 showed the highest AUCs (~0.75), while a combined model of all proteins yielded an AUC of ~0.76, indicating complementary rather than singularly dominant contributions.
Conclusion: These exploratory findings support further evaluation of circadian proteins as biomarkers in pediatric asthma and warrant investigation of chronotherapy in appropriately designed trials, rather than justifying a specific dosing time at present. Circadian proteins, particularly PER2 and BMAL1, may serve as potential biomarkers and support precision-based chronotherapy in pediatric asthma management.

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Introduction

Asthma is a chronic inflammatory disease affecting millions of children worldwide, characterized by airway hyperresponsiveness, reversible airflow obstruction, and chronic inflammation.[1] Despite advances in treatment, a significant proportion of pediatric patients experience poor asthma control, leading to frequent exacerbations, hospitalizations, and reduced quality of life.[2] While conventional asthma management focuses on bronchodilators and anti-inflammatory therapy, recent research suggests that circadian rhythms play a critical role in immune regulation and airway inflammation, influencing asthma severity and treatment response.[3]

Circadian rhythms are endogenous biological processes that regulate physiological functions over a 24-hour period, including pulmonary function, immune responses, and airway inflammation.[4] The molecular clockwork consists of a group of core circadian proteins, including BMAL1, CLOCK, CRY1, CRY2, PER1, and PER2, which coordinate cellular processes, immune signaling, and inflammatory responses.[5] Disruptions in circadian rhythms have been linked to increased airway inflammation, altered immune cell trafficking, and nocturnal asthma symptoms, which are commonly reported in children with asthma.[6,7]

Recent studies indicate that circadian clock dysregulation affects lung function, particularly in patients with nocturnal asthma, where airway hyperresponsiveness worsens at night.[8] This pattern suggests that time-of-day variations in inflammatory mediators and circadian-regulated gene expression may influence asthma pathophysiology.[9] Additionally, emerging research has proposed that optimizing medication timing, termed chronotherapy, may improve asthma control by aligning treatment administration with circadian variations in airway inflammation and glucocorticoid receptor sensitivity.[10]

Core clock proteins orchestrate inflammatory tone in the lung. The heterodimer CLOCK:BMAL1 activates E-box-driven transcription, thereby linking circadian timing to immune programs; loss or reduction of BMAL1 heightens allergic airway inflammation and amplifies virus-induced airway pathology.[8,11] Conversely, CRY1/CRY2 function as canonical repressors that dampen NF-κB/PKA signaling and constrain pro-inflammatory cytokine expression.[12] PER proteins—especially PER2—integrate epithelial inflammatory pathways and can protect lung tissue under stress.[13] Consistent with these mechanisms, the endogenous circadian system 'gates' airway responses, contributing to nocturnal worsening of asthma.[14] and several studies report altered expression of clock genes in people with asthma, with correlations to clinical features.[15]

Circadian disruption does not uniformly elevate clock proteins; instead, it reshapes phase, amplitude, and basal expression in a cell- and context-dependent manner. In asthma, airway epithelial datasets report altered—and in some cases reduced—expression of core clock genes such as PER2/PER3, with BMAL1 also reported lower in airway samples, while loss of CRY1/CRY2 de-represses inflammatory signaling.[12,16,17] Importantly, lung function exhibits an intrinsic circadian rhythm even in healthy individuals (e.g., FEV¤ tends to rise from morning to midday and decline later), while in asthma, the endogenous circadian system independently worsens nocturnal pulmonary function (lower FEV_1, higher airway resistance) beyond effects of sleep or behavior.[18,19] These nuances support evaluating multiple clock proteins rather than assuming a uniform directional change. At the same time, airway inflammation and hyperresponsiveness can disturb sleep, potentially reinforcing inflammation and symptoms—underscoring a bidirectional relationship.[14] These biological links provide a rationale for assessing BMAL1, CLOCK, CRY1, CRY2, PER1, and PER2 as candidate inflammatory biomarkers in pediatric asthma.[11,15]

Given the increasing evidence supporting the role of circadian rhythm proteins in asthma, this study aims to evaluate the association between circadian clock proteins and asthma control in children. By identifying biomarkers linked to circadian regulation, we aim to improve asthma management strategies and explore novel therapeutic approaches, such as precision-based chronotherapy.[20]

Materials and Methods

Study design and ethical approval

This study was designed as a cross-sectional, case-control investigation intended to compare circadian rhythm protein levels in children diagnosed with asthma and in healthy controls.

This study was approved by the Health Sciences University Hamidiye Scientific Research Ethics Committee (Date: November 14, 2024; No: 2024/13-13/2). Written informed consent was obtained from all participants and their legal guardians.

A post-hoc power analysis based on the observed effect size for BMAL1 (Cohen's d = 0.87) was conducted using GPower software, indicating that the current sample of 43 children with asthma and 34 healthy controls achieved a power of approximately 89% at an alpha level of 0.05.

Materials

Bovine serum albumin (BSA), Folin-Ciocalteu's phenol reagent, potassium phosphate monobasic (KH_2PO_4), and sodium potassium tartrate (NaK tartrate) were purchased from Sigma (St. Louis, MO, USA). Copper sulfate ($CuSO_4$), sodium hydroxide (NaOH), sodium carbonate anhydrous (Na_2CO_3), and sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$) were obtained from Riedel-de Haën (Germany). Human CRY1 and CRY2 ELISA kits were obtained from Elabscience (USA), Human PER1, PER2, and BMAL1 ELISA kits from BT LAB (UK), and the Human CLOCK ELISA kit from MyBioSource (USA).

Participants and inclusion/exclusion criteria

Children between the ages of 8 and 17 who had been followed in a pediatric allergy clinic for at least three visits, who had not experienced an acute asthma exacerbation or

used rescue medication in the previous month, and who satisfied the Global Initiative for Asthma (GINA) diagnostic criteria were included in the asthma group.[1] Asthma diagnosis was confirmed by evaluating clinical symptoms—namely, wheezing, shortness of breath, chest tightness, or cough that worsened at night or early in the morning—and by demonstrating an increase in FEV_1 of at least 12% and 200 mL after bronchodilator administration. Additional tests, such as exercise challenges or bronchial provocation, were conducted if necessary. Exclusion criteria included any neurologic, cardiac, metabolic, or psychiatric conditions; acute infection within the previous week; inability to perform spirometry; and the presence of comorbid atopic diseases such as allergic rhinitis, atopic dermatitis, or food allergy. The healthy control group consisted of children of similar age and sex distribution who did not have asthma or other chronic systemic diseases and had no significant comorbidities. A total of 43 children with asthma and 34 healthy controls were enrolled.

Pulmonary function testing

Pulmonary function tests were performed in accordance with the American Thoracic Society and European Respiratory Society guidelines using a calibrated spirometer.[21] Participants were seated in a well-ventilated room and instructed to take a deep breath and exhale forcefully into the spirometer for as long and as hard as possible. Each participant performed at least three acceptable forced vital capacity (FVC) maneuvers, and the best measurements were recorded for analysis. Parameters included forced expiratory volume in one second (FEV_1), the FEV_1/FVC ratio, peak expiratory flow (PEF), and mid-expiratory flow (MEF_{25-75}). All tests were supervised by a trained technician to ensure quality control and reproducibility.

Asthma control assessment

Asthma control was evaluated using the Asthma Control Test (ACT), which has been validated for use in the Turkish population.[22] The ACT consists of five questions that assess symptoms and the use of reliever medication over the preceding four weeks, yielding a score of 20–25 for well-controlled asthma, 15–19 for partially controlled asthma, and 5–14 for uncontrolled asthma. The questionnaire was administered to all participants on the same day as pulmonary function testing.

Sleep quality

Sleep quality was measured using the Children's Sleep Habits Questionnaire (CSHQ), a parent-reported instrument that examines key aspects of sleep, including bedtime resistance, nighttime awakenings, breathing problems during sleep, and morning wakefulness.[23] A total score of 41 or higher was considered indicative of poor sleep quality, whereas a score below 41 indicated good sleep quality.

Circadian rhythm protein measurement

Blood samples for circadian rhythm protein analysis were collected from all participants in the morning (08:00–10:00), preferably under fasting conditions. Because many inflammatory mediators and clock-regulated transcripts exhibit diurnal variation, a fixed morning window was used to limit time-of-day confounding and standardize pre-analytical conditions in this pediatric cohort.[24,25] Samples were centrifuged at 4 °C (10 min, 4500 rpm), serum was aliquoted, and stored at −80 °C for up to three months.[26] The Lowry method for protein determination was used to measure the total protein content of the specimens.[27] Total protein content was used to calculate the serum levels of circadian rhythm proteins. Serum concentrations of clock proteins (BMAL1, CLOCK, CRY1, CRY2, PER1, PER2) were measured by sandwich ELISA (per the manufacturer's instructions) using a 7-point calibration fitted with a 4-parameter logistic model. All samples and standards were run in duplicate; plates included a blank, a matrix-matched control, and two QC controls (low/high). Duplicate variability >15% CV triggered re-measurement. Inter- and intra-assay CVs across QC levels were <10% and <12%, respectively.

Total protein in each serum sample was quantified in duplicate by the Lowry method using bovine serum albumin standards. To reduce matrix-related variability, ELISA concentrations (after applying the appropriate dilution factor) were normalized to the sample's total protein and reported as pg per mg total protein (pg/mg)—that is, the ELISA-derived concentration per unit of total protein in the same sample. Values below the lower limit of quantification (LLOQ) were treated as missing and excluded from statistical analyses. Plate performance was monitored using QC controls, and no plate-wise correction was required.

Statistical analysis

All data were analyzed using JAMOVI (Version 2.6.26). Continuous variables are expressed as mean ± SD or median (IQR), depending on normality (Shapiro–Wilk). Group comparisons were performed using Student's *t*-test or Mann–Whitney *U* test, as appropriate; categorical variables were analyzed using x^2 or Fisher's exact tests. Group comparisons with more than two levels were analyzed using one-way ANOVA with post-hoc tests as appropriate. ROC curves and AUCs (95% CIs) were estimated for each protein. For the combined model, a multivariable logistic regression including all six proteins was fitted, and model-predicted probabilities were used to compute the combined ROC and AUC. Statistical significance was two-sided at $p<0.05$.

Results

Sociodemographic and clinical features of participants

Participants with asthma exhibited significantly poorer sleep quality compared to healthy controls, as indicated by

higher Children's Sleep Habits Questionnaire (CSHQ) scores (>41), with 51.2% of asthmatic children demonstrating poor sleep quality versus only 14.7% in the control group (p=0.001) (Table 1). Despite these notable differences in sleep quality, other sociodemographic characteristics—such as age, gender distribution, body weight, height, body mass index (BMI), and exposure to secondhand smoke—did not differ significantly between the asthma and control groups (p>0.05) (Table 1).

Sociodemographic and laboratory characteristics according to asthma control status

The sociodemographic and laboratory characteristics of participants based on asthma control status are summarized in Table 2. No statistically significant differences were observed between children with well-controlled asthma and those with partially controlled or uncontrolled asthma regarding age, sex, body weight, height, or body mass index (BMI) (all p>0.05). Likewise, total IgE levels and asthma follow-up durations did not differ significantly between groups (p=0.10 and p=0.78, respectively). Eosinophil counts were also similar between groups (p=0.79). However, children with partially controlled or uncontrolled asthma exhibited significantly lower values for FEV_1 (liters), FEV_1/FVC, PEF (both % and liters), and MEF_{25-75} (both % and liters) compared to well-controlled asthmatic children (p=0.004, p=0.006, p=0.04, p=0.01, p=0.03, and p=0.003, respectively) (Figure 1). Furthermore, poor sleep quality was significantly more common among children with partially controlled or uncontrolled asthma (p=0.001). Nighttime symptoms showed a near-significant difference between groups (p=0.07) (Table 2).

Table 1 Sociodemographic and Clinical Characteristics of Study Participants.

		Asthma n=43	Control n=34	p=
Sex	girls, n (%)	17 (39.5)	18 (52.9)	0.24[a]
Age	years (SD)	11.2 (2.36)	10.5 (2.97)	0.23[b]
Weight, (kg)	kg (SD)	41.8 (15.9)	43.3 (12.9)	0.65[b]
Height, (cm)	cm (SD)	144 (14.3)	146 (15.5)	0.59[b]
Body mass index	(SD)	19.5 (4.9)	19.9 (3.07)	0.88[b]
Secondhand smoke exposure*	yes, n (%)	13 (30.2)	9 (26.5)	0.68[a]
CSHQ scores	>41, n (%)	22 (51.2)	5 (14.7)	0.001[a]

()= Standard Deviation.
[a]Chi-square test.
[b]Student's t test.
CSHQ: Children's Sleep Habits Questionnaire.

Table 2 Sociodemographic and Laboratory Characteristics of Children with Asthma Based on Asthma Control Test Scores.

ACT		Well-Controlled (n=19)	Partially-controlled and Uncontrolled (n=24)	p=
Age	years (SD)	11.1 (2.68)	11.0 (2.12)	0.83[a]
Sex	girls, n (%)	6 (31.6)	11 (45.8)	0.31[b]
Weight	kg (SD)	44 (17.6)	38.3 (13.9)	0.26[a]
Height	cm (SD)	148 (18.5)	140 (9.32)	0.07[a]
Body mass index	(SD)	19.2 (4.13)	19.2 (5.40)	0.96[a]
Total IgE	kIU/l (SD)	85.9 (43.2)	140 (124)	0.10[a]
Asthma Follow-up Duration	months (SD)	10.7 (4.91)	11.1 (3.20)	0.78[a]
Eosinophil		313 (374)	342 (301)	0.79[a]
Secondhand smoke Exposure	yes, n (%)	5 (26.3)	8 (33.3)	0.62[b]
CSHQ scores	>41, n (%)	0 (0)	21 (87.5)	0.001[b]
Night Symptom	yes, n (%)	5 (26.3)	13 (41.9)	0.07[b]

()= Standard Deviation.
[a]Student's t test.
[b]Chi-square test.
CSHQ: Children's Sleep Habits Questionnaire.

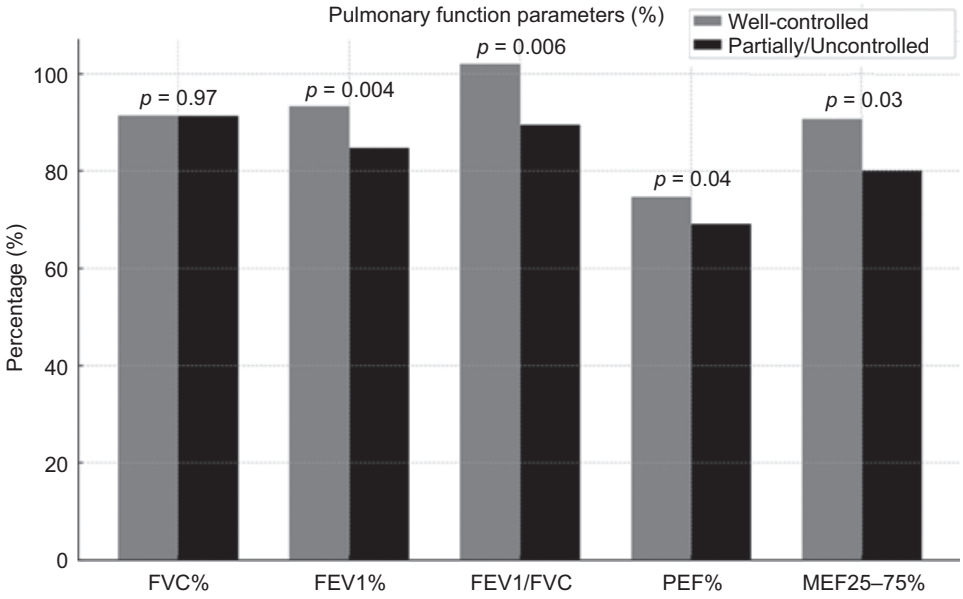

Figure 1 Comparison of Pulmonary Function Parameters by Asthma Control Status. Pulmonary function parameters (%) comparison between well-controlled and partially/uncontrolled asthmatic children. Significant differences were observed for FEV_1%, FEV_1/FVC, PEF%, and MEF_{25-75}% parameters. Values are presented as mean percentages (%). Statistical significance indicated by p-values placed above bars.

Table 3 Comparison of Circadian Rhythm Protein Levels Between Asthma and Control Groups. Units for proteins: pg/mg total protein.

	Asthma (n=43)			Control (n=34)			p=
	Mean	IQR	95% CI	Mean	IQR	95% CI	
BMAL1	1.20 (0.47)	0.69	1.06-1.35	0.81 (0.43)	0.51	0.66-0.96	<0.001[a]
CLOCK	0.48 (0.20)	0.13	0.42-0.55	0.38 (0.32)	0.21	0.27-0.50	<0.001[b]
CRY1	0.42 (0.21)	0.18	0.36-0.48	0.37 (0.37)	0.18	0.25-0.51	0.002[b]
CRY2	0.36 (0.23)	0.21	0.29-0.44	0.34 (0.35)	0.20	0.22-0.46	0.72[a]
PER1	0.42 (0.25)	0.29	0.34-0.50	0.29 (0.19)	0.14	0.22-0.35	0.008[a]
PER2	0.64 (0.26)	0.34	0.56-0.72	0.43 (0.22)	0.20	0.36-0.51	<0.001[a]

()= Standard Deviation.
[a]Student's t test ·
[b]Mann-Whitney U test.

Circadian rhythm protein levels in asthma

Asthmatic children displayed significantly elevated circadian rhythm proteins (BMAL1, CLOCK, CRY1, PER1, and PER2) compared to healthy controls (p<0.001, p<0.001, p=0.002, p=0.008, and p<0.001, respectively) (Table 3). However, CRY2 levels were similar between groups (p=0.72). These elevated protein levels suggest circadian dysregulation in children with asthma, potentially impacting airway inflammation and symptom severity.

Association between sleep quality and circadian rhythm proteins

Asthmatic children with poor sleep quality demonstrated significantly higher levels of the circadian rhythm proteins BMAL1, PER1, and PER2 compared to children with good sleep quality (p=0.005, p=0.01, and p<0.001, respectively) (Table 4). Although CLOCK protein levels exhibited a trend toward elevation in children with poor sleep quality, the difference was not statistically significant (p=0.30). CRY1 and CRY2 proteins were not significantly associated with sleep quality (p>0.05), indicating a selective impact of specific circadian proteins on sleep regulation.

Circadian rhythm proteins according to sleep quality in asthmatic patients

When circadian rhythm proteins were analyzed based on sleep quality (CSHQ scores), significantly higher levels of BMAL1, PER1, and PER2 were observed among asthmatic children with poor sleep quality (CSHQ>41) compared to those with good sleep quality (CSHQ<41) (p=0.03, p=0.01, and p=0.02, respectively) (Table 5). However, no significant

Table 4 Circadian Rhythm Proteins in Asthmatic and Control Groups Based on Children's Sleep Habits Questionnaire (CSHQ Scores).

	Asthma (n=43)		Control (n=34)		p=
	<41	>41	<41	>41	
BMAL1	1.02 (0.38)	1.38 (0.49)	0.80 (0.46)	0.87 (0.29)	0.005[a]
CLOCK	0.47 (0.21)	0.50 (0.20)	0.37 (0.30)	0.46 (0.44)	0.14[a]
CRY1	0.41 (0.22)	0.44 (0.18)	0.36 (0.35)	0.47 (0.51)	0.53[a]
CRY2	0.36 (0.21)	0.37 (0.26)	0.33 (0.34)	0.39 (0.44)	0.73[a]
PER1	0.31 (0.15)	0.53 (0.29)	0.30 (0.20)	0.24 (0.01)	0.01[a]
PER2	0.54 (0.22)	0.73 (0.26)	0.46 (0.24)	0.38 (0.01)	<0.001[a]

[a]one-way ANOVA.
()= Standard Deviation.

Table 5 Comparison of Circadian Rhythm Proteins Among Asthmatic Children Based on Sleep Quality (CSHQ Scores).

	<41 (n=21)			>41 (n=22)			p=
	Mean	IQR	95% CI	Mean	IQR	95% CI	
BMAL1	1.02 (0.38)	0.59	0.84-1.19	1.38 (0.49)	0.88	1.16-1.60	0.03[a]
CLOCK	0.47 (0.21)	0.10	0.37-0.57	0.50 (0.21)	0.17	0.41-0.60	0.67[b]
CRY1	0.41 (0.22)	0.19	0.31-0.51	0.44 (0.18)	0.12	0.36-0.52	0.63[b]
CRY2	0.36 (0.21)	0.22	0.26-0.45	0.37 (0.26)	0.19	0.26-0.49	0.88[b]
PER1	0.31 (0.15)	0.16	0.25-0.38	0.53 (0.29)	0.40	0.39-0.66	0.01[a]
PER2	0.54 (0.22)	0.21	0.44-0.64	0.73 (0.26)	0.30	0.61-0.85	0.02[b]

()= Standard Deviation.
[a]Mann-Whitney U.
[b]Student's t test.

differences were found in CLOCK, CRY1, or CRY2 proteins according to sleep quality status ($p>0.05$). These results suggest a potential link between circadian rhythm disruptions and impaired sleep quality in pediatric asthma.

Circadian rhythm proteins and asthma control status

Significant associations were identified between asthma control and specific circadian rhythm proteins. Children with partially controlled or uncontrolled asthma had elevated levels of BMAL1, PER1, and PER2 proteins compared to well-controlled asthma patients ($p=0.04$, $p=0.04$, and $p=0.006$, respectively) (Table 6). In contrast, CLOCK, CRY1, and CRY2 proteins did not differ significantly according to asthma control status ($p>0.05$). These findings indicate the potential utility of specific circadian proteins as biomarkers for asthma severity and control.

ROC analysis of circadian rhythm proteins

ROC analysis revealed varying diagnostic performances of circadian rhythm proteins as biomarkers distinguishing asthmatic children from controls. Among single markers,

Table 6 Circadian Rhythm Protein Levels According to Asthma Control Test (ACT) Scores.

	Well-Controlled (n=19)			Partially-controlled – Uncontrolled (n=24)			p=
	Mean	IQR	95% CI	Mean	IQR	95% CI	
BMAL1	0.99 (0.35)	0.60	0.82-1.16	1.37 (0.50)	0.84	1.16-1.58	0.02[a]
CLOCK	0.48 (0.22)	0.11	0.37-0.58	0.49 (0.19)	0.14	0.40-0.57	0.81[b]
CRY1	0.42 (0.22)	0.19	0.31-0.53	0.43 (0.18)	0.13	0.34-0.50	0.99[b]
CRY2	0.35 (0.23)	0.24	0.23-0.46	0.38 (0.23)	0.18	0.28-0.48	0.67[b]
PER1	0.32 (0.15)	0.24	0.24-0.39	0.50 (0.29)	0.42	0.37-0.63	0.04[a]
PER2	0.54 (0.20)	0.28	0.44-0.64	0.72 (0.28)	0.37	0.60-0.83	0.03[b]

()= Standard Deviation.
[a]Mann-Whitney U.
[b]Student's t test.

PER2 and BMAL1 provided the strongest individual discrimination between asthma and controls (AUCs ≈0.75; $p<0.01$ vs. 0.5), whereas a multivariable model including all six proteins achieved a slightly higher AUC (~0.76). These differences should be interpreted as descriptive, as the study was not designed or powered to test head-to-head superiority among proteins. Lower diagnostic performances were noted for CRY1 (AUC=0.70; SE=0.06; 95% CI: 0.58–0.82) and PER1 (AUC=0.68; SE=0.06; 95% CI: 0.56–0.80). CRY2 exhibited the lowest diagnostic performance (AUC=0.63; SE=0.06; 95% CI: 0.51–0.75) (Figure 2).

Discussion

Our study identified significant elevations in circadian rhythm proteins (BMAL1, CLOCK, CRY1, PER1, and PER2) among children with asthma compared to healthy controls, particularly highlighting the roles of BMAL1, PER1, and PER2 in relation to asthma control and sleep quality. These findings underscore the potential involvement of circadian dysregulation in the pathogenesis and symptom severity of pediatric asthma.

Elevated circadian rhythm proteins, especially PER2 and BMAL1, may reflect enhanced inflammatory signaling pathways in asthmatic patients. Previous studies suggest that circadian proteins modulate immune responses by regulating inflammatory cytokines and chemokines, which directly influence airway inflammation and bronchial hyperreactivity.[28,29] Specifically, alterations in the expression of PER2 and BMAL1 have been linked to increased Th2 cytokine production, thereby enhancing airway inflammation and asthma severity.[30]

Our findings highlight heterogeneity and complementarity across clock proteins. While PER2 and BMAL1 performed best as single markers in this cohort, the combined model performed slightly better, arguing against the mechanistic primacy of any one protein and in favor of multi-analyte panels. Previous studies have demonstrated similar associations between elevated PER2 and BMAL1 levels and nocturnal asthma symptoms in children.[31] However, our finding that CRY2 protein levels do not differ significantly between asthmatic and control groups diverges from earlier reports, indicating the need for further investigation into protein-specific roles in asthma pathophysiology.[32]

Contemporary guidelines do not recommend a specific dosing time for daily controller therapy, focusing instead on the choice of an ICS-containing regimen and adherence.[1] While emerging studies suggest that the timing of inhaled corticosteroids can influence nocturnal control, the evidence base remains limited, heterogeneous, and largely not pediatric-specific; thus, any timing adjustments should be individualized and undertaken within guideline-directed care. Chronotherapy may improve therapeutic efficacy by aligning anti-inflammatory and bronchodilator treatments with peak inflammatory activity and periods of bronchial hyperresponsiveness.[10] Chronotherapy refers to aligning treatment with the body's endogenous circadian rhythms

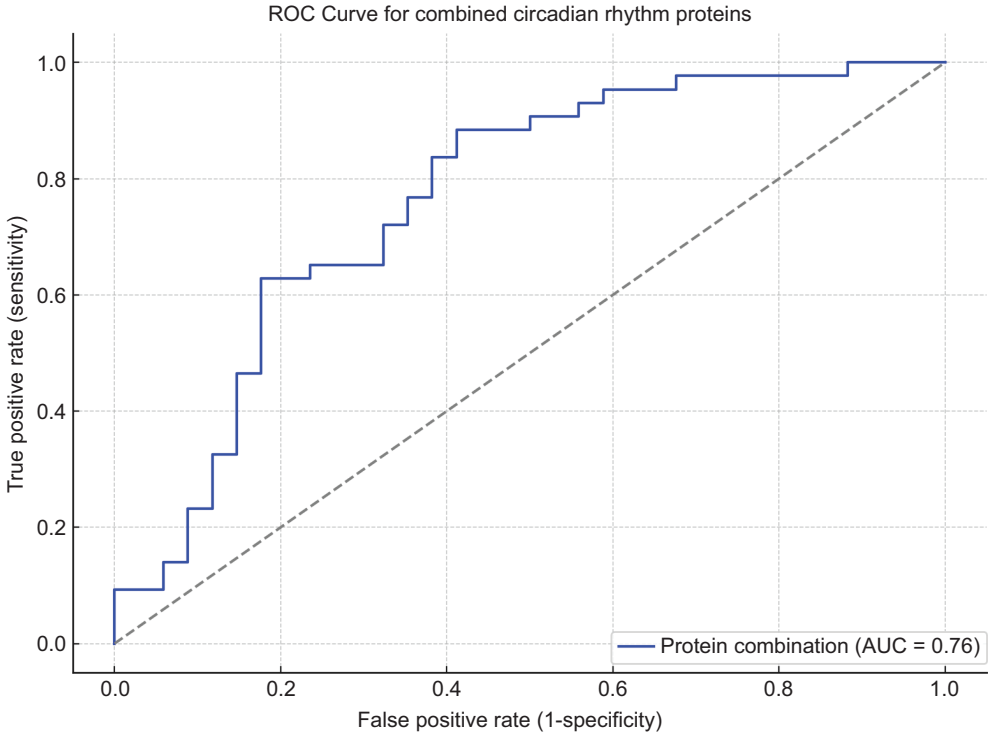

Figure 2 ROC Curve of Combined Circadian Rhythm Proteins in Differentiating Asthmatic Children from Controls. The ROC curve illustrates the diagnostic performance of the combined circadian rhythm proteins (CRY1, CRY2, PER1, PER2, CLOCK, BMAL1) in distinguishing between asthmatic and control groups. The combined protein model achieved an AUC of 0.76 (Standard Error [SE]=0.05; 95% Confidence Interval [CI]: 0.66-0.86), indicating good discriminative ability. The diagonal dashed line represents random classification (reference line).

to improve efficacy and/or reduce adverse effects.[33] In asthma, circadian biology modulates airway tone and pulmonary function, motivating interest in whether the time of day of inhaled corticosteroid (ICS) dosing affects control.[18] Evidence is mixed: a meta-analysis and several trials suggest that evening or mid-afternoon once-daily ICS dosing can improve nocturnal lung function compared with morning dosing in selected adult cohorts, whereas other studies report no meaningful difference between morning and evening administration.[34,36] Importantly, contemporary GINA reports do not recommend a specific dosing time; emphasis remains on using an ICS-containing regimen and ensuring adherence.[1] Consistent with this landscape, our findings are hypothesis-generating and support further investigation of chronotherapy rather than a change to routine prescribing. Monitoring circadian biomarkers such as PER2 and BMAL1 may therefore provide valuable information for personalized asthma management and improved clinical outcomes.

A major strength of our study is the use of standardized pulmonary function testing and the validated Asthma Control Test to objectively assess asthma control. Additionally, rigorous statistical methods, including ROC analysis, strengthened our biomarker evaluation. Nevertheless, our study has limitations, including its cross-sectional design, which restricts causal inference. Furthermore, the relatively small sample size may limit the generalizability of the findings, necessitating validation in larger, prospective cohorts.

This study focuses on the mechanisms underlying diseases, a major concern in toxicology. Our findings provide new insights into asthma pathophysiology and offer innovative treatment strategies. While inhaled corticosteroids are commonly used to manage asthma and are effective in controlling the disease, their long-term use can cause various side effects. Diseases can directly affect the circadian rhythm; furthermore, commonly prescribed medications may exacerbate disruptions in circadian rhythm protein levels.[26] Considering the adverse effects of asthma medications, particularly on sleep quality, the demand for alternative treatment approaches is increasing. In this context, the role of circadian rhythm proteins in asthma etiology, as presented in this study, is noteworthy. In the future, evaluating the therapeutic potential of synthetic derivatives of these proteins may contribute to the development of biological rhythm-compatible treatment options.

Future research should focus on longitudinal studies examining how circadian protein levels influence long-term asthma control, medication responsiveness, and disease progression across diverse pediatric populations. Randomized controlled trials investigating chronotherapeutic interventions could provide critical evidence to support precision-based treatment approaches in pediatric asthma.

In conclusion, this study highlights significant disruptions in circadian rhythm proteins—particularly elevated BMAL1, PER1, and PER2 levels—in children with asthma compared to healthy controls. These alterations correlate with poorer asthma control, decreased pulmonary function, and impaired sleep quality, underscoring the potential role of circadian dysregulation in pediatric asthma pathogenesis. Among the investigated biomarkers, PER2 and BMAL1 demonstrated strong discriminative performance, indicating their potential utility as biomarkers for diagnosing and monitoring pediatric asthma. Our findings support further exploration of chronotherapy, suggesting that aligning asthma treatment with circadian rhythms may optimize therapeutic efficacy and improve clinical outcomes in children with asthma. Future longitudinal studies and clinical trials focusing on circadian-based therapeutic interventions are warranted to validate these findings and enhance their applicability within precision medicine approaches for pediatric asthma management.

Author's Contributions

AY: Conceptualization, methodology, data analysis, manuscript drafting. FKÇ: Laboratory analysis, data interpretation. TA: Patient recruitment, clinical data collection. DB: Literature review, manuscript editing. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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