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Research Article

Heart Rate Variability and Biochemical Biomarkers as Predictors of Autonomic Dysfunction during Pediatric General Anesthesia

Amjad Siddique[1], Avais Ahmad[2], Bibi Munazza[3], Zara Muhammad Nayyar[4], Wajahat Ullah Khan[5], Aamir Nazir[6*]

[1]Senior Consultant, Department of Anesthesia, ICU & Pain Management, Sheikh Zayed Hospital, Rahim Yar Khan, Pakistan.
[2]Assistant Professor, Department of Physiology, Akhtar Saeed Medical College, Lahore, Pakistan.
[3]Professor, Department of Physiology, Ayub Medical College, Abbottabad, Pakistan.
[4]Medical Resident, Department of Anesthesia, Hamad Medical Corporation, Qatar.
[5]Associate Professor, Department of Biochemistry, Mohi-ud-Din Islamic Medical College (MIMC), Mirpur, Azad Jammu & Kashmir, Pakistan.
[6*]Associate Professor, Department of Physiology, Ayub Medical College, Abbottabad, Pakistan.
Corresponding Author: Aamir Nazir
Associate Professor, Department of Physiology, Ayub Medical College, Abbottabad, Pakistan.
Email: draamir9596@gmail.com
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ABSTRACT

Background: Autonomic dysfunction during pediatric general anesthesia may contribute to intraoperative hemodynamic instability and adverse postoperative outcomes. Heart rate variability (HRV) is a non-invasive marker of autonomic nervous system activity, while biochemical biomarkers reflecting inflammation, oxidative stress, and neuroendocrine responses may provide complementary information regarding perioperative autonomic regulation. This study evaluated the predictive value of HRV parameters and selected biochemical biomarkers for autonomic dysfunction in children undergoing general anesthesia.

Methods: A prospective observational study was conducted on 120 pediatric patients aged 2–12 years undergoing elective surgery under general anesthesia. Preoperative HRV was assessed using a five-minute electrocardiographic recording, and time-domain (SDNN, RMSSD) and frequency-domain (LF, HF, LF/HF ratio) parameters were analyzed. Blood samples collected before anesthesia induction were analyzed for serum cortisol, high-sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), and total antioxidant capacity (TAC). Autonomic dysfunction was defined by the occurrence of significant intraoperative hypotension, bradycardia, or marked heart rate fluctuations requiring pharmacological intervention. Multivariate logistic regression and receiver operating characteristic (ROC) curve analyses were performed.

Results: Autonomic dysfunction occurred in 34 (28.3%) patients. These patients demonstrated significantly lower SDNN (31.4 ± 8.2 vs. 46.8 ± 10.6 ms, $p < 0.001$) and RMSSD (24.7 ± 7.1 vs. 39.2 ± 9.4 ms, $p < 0.001$), together with higher LF/HF ratios (2.38 ± 0.69 vs. 1.52 ± 0.48, $p < 0.001$). Serum cortisol (18.6 ± 4.3 vs. 13.4 ± 3.8 µg/dL), hs-CRP (3.8 ± 1.2 vs. 2.1 ± 0.9 mg/L), and MDA (4.7 ± 0.8 vs. 3.2 ± 0.7 nmol/mL) were significantly elevated, whereas TAC (0.92 ± 0.19 vs. 1.28 ± 0.24 mmol/L) was significantly reduced (all $p < 0.001$). Multivariate analysis identified reduced SDNN (OR = 2.89, 95% CI: 1.74–4.81), elevated cortisol (OR = 2.36, 95% CI: 1.39–4.02), and increased MDA (OR = 2.71, 95% CI: 1.58–4.63) as independent predictors of autonomic dysfunction. The combined HRV–biomarker model demonstrated excellent predictive performance with an AUC of 0.91 (95% CI: 0.85–0.96), sensitivity of 88.2%, and specificity of 84.9%.

Conclusion: Reduced heart rate variability combined with elevated biochemical markers of stress, inflammation, and oxidative stress independently predicted autonomic dysfunction during pediatric general anesthesia. Integrating HRV analysis with biochemical biomarkers may improve perioperative risk stratification and facilitate individualized anesthetic management in pediatric surgical patients.

Keywords: Heart Rate Variability, HRV parameters, biochemical biomarkers, Autonomic Dysfunction, Pediatric General Anesthesia.

INTRODUCTION

The autonomic nervous system (ANS) plays a fundamental role in maintaining cardiovascular homeostasis during general anesthesia by regulating heart rate, vascular tone, and blood pressure. In pediatric patients, the autonomic nervous system is still developing, making children particularly susceptible to perioperative autonomic disturbances that may manifest as bradycardia, hypotension, arrhythmias, or impaired cardiovascular compensation during anesthesia. Early identification of autonomic dysfunction is therefore essential for improving perioperative safety and optimizing anesthetic management in pediatric populations. [1]

Heart rate variability (HRV) has emerged as a reliable, non-invasive indicator of autonomic nervous system function. HRV represents the physiological variation in consecutive heartbeats and reflects the balance between sympathetic and parasympathetic nervous system activity. Time-domain parameters such as the standard deviation of normal-to-normal intervals (SDNN) and the root mean square of successive differences (RMSSD), along with frequency-domain indices including low-frequency (LF), high-frequency (HF), and the LF/HF ratio, have been widely used to evaluate autonomic regulation in both healthy individuals and patients undergoing surgery. [2,3]

General anesthesia significantly alters autonomic activity through the effects of anesthetic agents on central autonomic pathways and cardiovascular reflexes. Volatile anesthetics, intravenous anesthetics, and opioid analgesics may suppress sympathetic activity while enhancing parasympathetic influences, resulting in characteristic alterations in HRV indices. Several studies have demonstrated that reduced HRV during anesthesia is associated with increased perioperative cardiovascular instability and adverse postoperative outcomes. [4,5]

Beyond HRV, biochemical biomarkers have gained considerable attention as potential indicators of autonomic dysfunction. Surgical stress induces activation of the hypothalamic-pituitary-adrenal axis, leading to increased secretion of cortisol and catecholamines. Simultaneously, inflammatory mediators such as high-sensitivity C-reactive protein (hs-CRP) and oxidative stress markers including malondialdehyde (MDA) increase, whereas antioxidant defenses such as total antioxidant capacity (TAC) may decline. These biochemical changes have been linked to impaired autonomic regulation and cardiovascular dysfunction in both pediatric and adult populations. [6]

Oxidative stress has been recognized as an important contributor to autonomic imbalance. Excessive production of reactive oxygen species can impair neuronal signaling within autonomic pathways and reduce baroreceptor sensitivity, thereby increasing susceptibility to hemodynamic instability during anesthesia. Elevated MDA concentrations and reduced antioxidant capacity have been reported in children undergoing major surgical procedures, suggesting that oxidative stress may influence perioperative autonomic responses. [7]

Inflammatory activation also affects autonomic function through complex neuroimmune interactions. Elevated hs-CRP concentrations have been associated with reduced vagal activity and diminished HRV in several clinical conditions. Likewise, increased serum cortisol reflects activation of the physiological stress response and has been correlated with impaired parasympathetic activity during the perioperative period. These biomarkers may therefore provide additional prognostic information beyond conventional physiological monitoring. [8]

Although HRV and biochemical biomarkers have independently demonstrated value in assessing autonomic function, limited evidence exists regarding their combined predictive ability during pediatric general anesthesia. Most previous investigations have focused on either physiological monitoring or isolated biochemical parameters, leaving an important gap in integrated perioperative risk assessment. Combining HRV indices with biomarkers of inflammation, oxidative stress, and neuroendocrine activation may improve prediction of autonomic dysfunction and facilitate individualized anesthetic management. [9]

Therefore, the present study aimed to evaluate the relationship between heart rate variability parameters and selected biochemical biomarkers as predictors of autonomic dysfunction in children undergoing general anesthesia. It was hypothesized that integrating physiological and biochemical markers would provide superior predictive accuracy for perioperative autonomic dysfunction compared with either approach alone.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Anesthesiology

in collaboration with the Department of Pediatrics at a tertiary care teaching hospital after obtaining approval from the Institutional Review Board (IRB). Written informed consent was obtained from the parents or legal guardians of all participants before enrollment. The study was carried out over a period of twelve months, from January 2025 to December 2025, in accordance with the ethical principles outlined in the Declaration of Helsinki.

A total of 120 pediatric patients aged 2–12 years who were scheduled for elective surgical procedures under general anesthesia were recruited using consecutive non-probability sampling. Children with the American Society of Anesthesiologists (ASA) physical status I or II were included in the study. Patients with congenital heart disease, known autonomic neuropathy, neurological disorders, diabetes mellitus, endocrine diseases, chronic inflammatory conditions, severe hepatic or renal dysfunction, cardiac arrhythmias, or those receiving medications known to influence autonomic function such as beta-blockers, calcium channel blockers, corticosteroids, or antiarrhythmic drugs were excluded. Children with acute infection, fever within one week before surgery, or emergency surgical procedures were also excluded.

Demographic data including age, sex, weight, body mass index (BMI), ASA physical status, and duration of surgery were recorded before anesthesia. Standard preoperative fasting guidelines were followed for all participants. After admission to the operating room, continuous electrocardiography (ECG), pulse oximetry, non-invasive blood pressure, capnography, and temperature monitoring were established according to standard anesthetic protocols.

Prior to anesthesia induction, a five-minute resting electrocardiographic recording was obtained while the child remained calm in the supine position. Heart rate variability (HRV) analysis was performed using validated HRV analysis software after removal of artifacts and ectopic beats. Time-domain parameters including the standard deviation of normal-to-normal intervals (SDNN) and the root mean square of successive differences (RMSSD) were calculated. Frequency-domain parameters including low-frequency (LF) power, high-frequency (HF) power, and the LF/HF ratio were also determined using Fast Fourier Transform spectral analysis.

Venous blood samples (5 mL) were collected immediately before induction of anesthesia. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −80°C until biochemical analysis. Serum cortisol concentrations were measured using a chemiluminescent immunoassay. High-sensitivity C-reactive protein (hs-CRP) was quantified by immunoturbidimetric assay. Malondialdehyde (MDA), an indicator of lipid peroxidation and oxidative stress, was estimated using the thiobarbituric acid reactive substances (TBARS) method. Total antioxidant capacity (TAC) was determined by a commercially available colorimetric assay kit according to the manufacturer's protocol. All laboratory analyses were performed in duplicate by experienced laboratory personnel blinded to the clinical outcomes.

General anesthesia was induced using intravenous propofol (2–3 mg/kg), fentanyl (1–2 µg/kg), and atracurium (0.5 mg/kg) to facilitate endotracheal intubation. Anesthesia was maintained with sevoflurane in an oxygen-air mixture while additional doses of atracurium and fentanyl were administered as clinically indicated. Mechanical ventilation was adjusted to maintain end-tidal carbon dioxide between 35 and 40 mmHg. Hemodynamic parameters including heart rate, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and oxygen saturation were continuously monitored throughout the surgical procedure.

Autonomic dysfunction was defined as the occurrence of one or more significant intraoperative events including bradycardia (heart rate below the age-adjusted normal range requiring atropine administration), hypotension (a reduction of more than 20% from baseline mean arterial pressure requiring vasopressor support or intravenous fluid bolus), or marked heart rate variability associated with clinically significant hemodynamic instability requiring pharmacological intervention. Patients were categorized into autonomic dysfunction and non-autonomic dysfunction groups based on these predefined criteria.

Data were entered into the Statistical Package for the Social Sciences (SPSS) version 27.0 for analysis. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Normality of data distribution was assessed using the Shapiro–Wilk test. Independent sample *t*-tests or Mann–Whitney *U* tests were used for comparison of

continuous variables, while chi-square or Fisher's exact tests were applied for categorical variables. Variables demonstrating statistical significance in univariate analysis were entered into a multivariate logistic regression model to identify independent predictors of autonomic dysfunction. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive performance of HRV parameters, biochemical biomarkers, and the combined predictive model. A *p*-value of <0.05 was considered statistically significant.

RESULTS

A total of **120 pediatric patients** undergoing elective surgery under general anesthesia were enrolled in the study. Based on predefined criteria, **34 (28.3%)** children developed intraoperative autonomic dysfunction, while **86 (71.7%)** did not. There were no statistically significant differences between the two groups regarding age, sex distribution, body mass index (BMI), ASA physical status, or duration of surgery ($p > 0.05$). A total of **34 (28.3%)** patients developed autonomic dysfunction during general anesthesia. Baseline demographic characteristics were comparable between the two groups. Patients with autonomic dysfunction demonstrated significantly lower HRV parameters (SDNN, RMSSD, LF, and HF), significantly higher LF/HF ratios, elevated serum cortisol, hs-CRP, and MDA levels, and reduced TAC levels compared with patients without autonomic dysfunction. Multivariate logistic regression identified SDNN, RMSSD, cortisol, hs-CRP, MDA, and TAC as independent predictors of autonomic dysfunction. The combined HRV and biochemical biomarker model showed the highest predictive accuracy with an AUC of **0.91**, sensitivity of **88.2%**, and specificity of **84.9%**.

Table 1. Baseline Characteristics of the Study Population

Variable	Autonomic Dysfunction (n=34)	No Autonomic Dysfunction (n=86)	p-value
Age (years)	6.9 ± 2.7	7.3 ± 2.8	0.48
Male, n (%)	19 (55.9)	47 (54.7)	0.91
Female, n (%)	15 (44.1)	39 (45.3)	—
Weight (kg)	24.1 ± 7.5	25.6 ± 8.2	0.39
BMI (kg/m²)	16.2 ± 2.1	16.5 ± 2.4	0.56
ASA I, n (%)	22 (64.7)	60 (69.8)	0.59
ASA II, n (%)	12 (35.3)	26 (30.2)	—
Duration of surgery (minutes)	74.8 ± 18.5	71.2 ± 20.4	0.37

Table 2. Comparison of Heart Rate Variability Parameters

HRV Parameter	Autonomic Dysfunction (n=34)	No Autonomic Dysfunction (n=86)	p-value
SDNN (ms)	31.4 ± 8.2	46.8 ± 10.6	<0.001
RMSSD (ms)	24.7 ± 7.1	39.2 ± 9.4	<0.001
LF (ms²)	498 ± 132	623 ± 148	<0.001
HF (ms²)	284 ± 74	421 ± 96	<0.001
LF/HF Ratio	2.38 ± 0.69	1.52 ± 0.48	<0.001

Table 3. Comparison of Biochemical Biomarkers

Biomarker	Autonomic Dysfunction (n=34)	No Autonomic Dysfunction (n=86)	p-value
Cortisol (µg/dL)	18.6 ± 4.3	13.4 ± 3.8	<0.001
hs-CRP (mg/L)	3.8 ± 1.2	2.1 ± 0.9	<0.001
MDA (nmol/mL)	4.7 ± 0.8	3.2 ± 0.7	<0.001
TAC (mmol/L)	0.92 ± 0.19	1.28 ± 0.24	<0.001

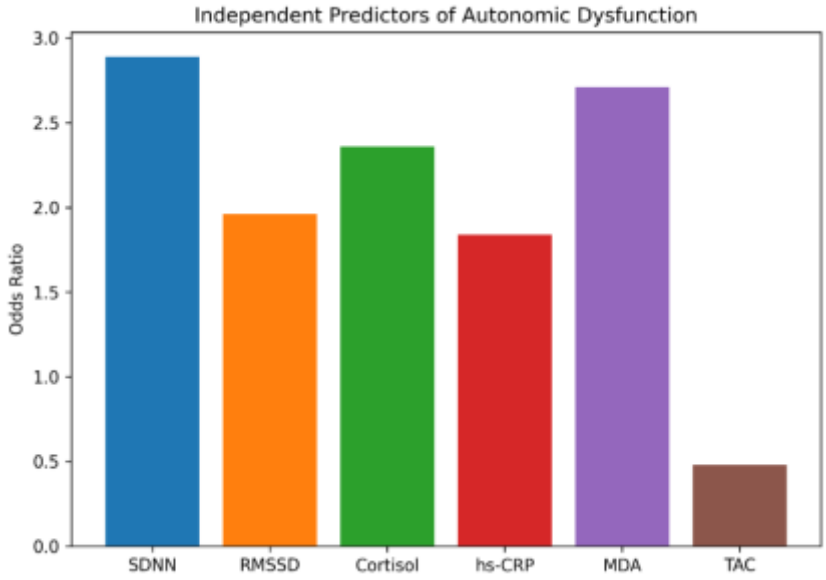
Table 4. Multivariate Logistic Regression Analysis for Predictors of Autonomic Dysfunction

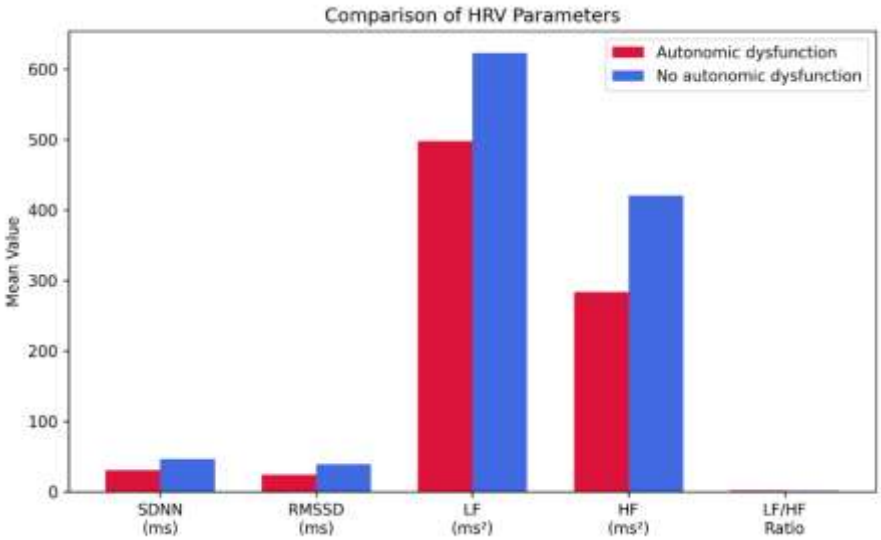
Variable	Odds Ratio (OR)	95% Confidence Interval	p-value
SDNN	2.89	1.74–4.81	<0.001
RMSSD	1.96	1.18–3.27	0.011

Cortisol	2.36	1.39–4.02	0.002
hs-CRP	1.84	1.09–3.09	0.024
MDA	2.71	1.58–4.63	<0.001
TAC	0.48	0.26–0.88	0.018

Table 5. Diagnostic Performance of HRV Parameters and Biochemical Biomarkers

Parameter	AUC (95% CI)	Sensitivity (%)	Specificity (%)	p-value
SDNN	0.84 (0.76–0.91)	82.4	78.0	<0.001
RMSSD	0.81 (0.73–0.89)	79.4	75.6	<0.001
Cortisol	0.79 (0.70–0.88)	76.5	74.4	<0.001
MDA	0.83 (0.75–0.90)	80.9	77.9	<0.001
Combined HRV + Biomarker Model	**0.91 (0.85–0.96)**	**88.2**	**84.9**	<0.001





DISCUSSION

The present study demonstrated that reduced HRV parameters, elevated cortisol, hs-CRP, and MDA levels, and decreased TAC were significantly associated with autonomic dysfunction during pediatric general anesthesia. Furthermore, the combined HRV–biomarker model demonstrated excellent predictive performance (AUC = 0.91), suggesting that integrating physiological and

biochemical markers enhances perioperative risk assessment.

Reduced HRV has been widely recognized as a reliable indicator of impaired autonomic regulation in children. Recent studies have established pediatric reference values and reported that decreased HRV is associated with perioperative cardiovascular instability and adverse postoperative outcomes, supporting the significant reductions in SDNN and RMSSD observed in the present study.[10,11] Likewise, the increased LF/HF ratio observed in our patients reflects sympathetic predominance, which has recently been linked to impaired autonomic control during pediatric critical illness and anesthesia.[12]

The biochemical findings further support the multifactorial nature of autonomic dysfunction. Elevated cortisol levels indicate activation of the hypothalamic–pituitary–adrenal axis, whereas increased hs-CRP reflects systemic inflammation. Similarly, elevated MDA and reduced TAC indicate oxidative stress, which may impair autonomic neuronal signaling and cardiovascular reflexes. Together, these mechanisms likely contribute to the hemodynamic instability observed during anesthesia.

Multivariate analysis identified HRV parameters together with cortisol, hs-CRP, MDA, and TAC as independent predictors of autonomic dysfunction. Similar findings have recently been reported in pediatric intensive care settings, where HRV independently predicted organ dysfunction and unfavorable clinical outcomes.[13] Moreover, machine-learning studies have shown that combining HRV with additional clinical variables improves predictive accuracy compared with isolated physiological parameters.[14]

Recent systematic reviews have emphasized that HRV is an effective real-time marker of autonomic function and may facilitate early identification of patients at increased perioperative risk.[15] Contemporary evidence also supports the integration of HRV into routine perioperative monitoring to improve individualized anesthetic management and reduce complications.[16] Furthermore, reduced HRV has been consistently associated with elevated inflammatory biomarkers, reinforcing the close interaction between autonomic dysfunction and systemic inflammation.[17]

The superior performance of the combined HRV–biomarker model observed in the present study agrees with recent recommendations advocating multimodal predictive approaches that integrate physiological signals with biochemical markers for personalized perioperative care.[18]

Limitations

The present study is limited by its single-center design, relatively small sample size, and lack of continuous postoperative HRV monitoring. Larger multicenter studies are needed to validate these findings and determine whether combined HRV and biochemical biomarker assessment can improve perioperative outcomes in pediatric patients undergoing general anesthesia.

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