narra j

Original Article

Effect of the modified Atkins diet on NLRP3, caspase-1, IL-1β, and IL-10 in patients with tetralogy of Fallot undergoing open-heart surgery: A randomized controlled trial

Piprim B. Yanuarso[1,2*], Mulyadi M. Djer[1,2], Aryono Hendarto[1,2], Antonius H. Pudjiadi[1,2], Lisnawati Rachmadi[3,4], Heri Wibowo[5], Najib Advani[1,2], Indah K. Murni[6,7], Aria Kekalih[8,9], Rubiana Sukardi[10], Ismail Dilawar[11], Dhama S. Susanti[10] and Novianti Supriatna[12]

[1]Department of Child and Health, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [2]Department of Child and Health, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [4]Department of Anatomical Pathology, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [5]Integrated Laboratory for Diagnostic and Research Center, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [6]Department of Child and Health, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; [7]Department of Child and Health, Dr. Sardjito Hospital, Yogyakarta, Indonesia; [8]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [9]Department of Community Medicine, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [10]Integrated Cardiac Center, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia; [11]Department of Cardiothoracic Surgery, Jakarta Heart Center Hospital, Jakarta, Indonesia; [12]Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

*Corresponding author: basarahpiprim@gmail.com

Abstract

Cardiopulmonary bypass in tetralogy of Fallot (TOF) corrective surgery induces hyperinflammation by activating NLRP3, caspase-1, and interleukin-1β (IL-1β), subsequently triggering an interleukin-10 (IL-10) response. Despite its known metabolic and anti-inflammatory effects, the impact of the modified Atkins diet (MAD) in pediatric cardiac surgery remains unexplored, with no studies on its use in TOF patients undergoing open-heart surgery. The aim of this study was to assess the effect of MAD on the expression of NLRP3, caspase-1, IL-1β, and IL-10, in TOF patients undergoing open-heart surgery. A double-arm, randomized-controlled trial was conducted with 44 TOF patients. The treatment group (n=22) received the MAD, a low-carbohydrate, high-fat regimen with unrestricted fat and protein intake for at least 14 days preoperatively, while the control group (n=22) followed a standard diet without carbohydrate restriction. Blood plasma and infundibulum heart tissues were collected for analysis. Whole blood samples were collected using a winged infusion needle before the intervention, an Abbocath infusion needle after 14 days of intervention, and a syringe without a needle connected to an arterial line in patients undergoing open-heart surgery at 6, 24, and 48 hours post-surgical correction. Infundibulum heart tissues were collected during the open-heart surgery. This study demonstrated significant differences in NLRP3 protein expression (p=0.015), caspase-1 protein expression (p=0.001), and IL-10 levels between after intervention and 6-, 24-, and 48-hours post-surgery in the MAD group compared to the control group. In contrast, no significant differences in IL-10 levels were observed in the control group between after intervention and 48 hours post-surgery (p=0.654). In conclusion, MAD may modulate perioperative inflammation in TOF patients undergoing open-heart surgery by downregulating NLRP3 and caspase-1 expression while sustaining IL-10 levels. Despite reduced NLRP3 and caspase-1 expression, unchanged IL-1β levels indicate alternative regulatory mechanisms.

Keywords: Ketogenic diet, modified Atkins diet, tetralogy of Fallot, NLRP3, cytokines

Received: January 17, 2025 - Accepted: March 15, 2025 - Published online: March 24, 2025

Introduction

Congenital heart disease (CHD) is the leading cause of mortality among congenital disorders in Indonesia, with an incidence of 8 per 1,000 live births [1]. Among various CHD subtypes, tetralogy of Fallot (TOF) is the most prevalent cyanotic CHD, characterized by right ventricular outflow tract obstruction, ventricular septal defect, overriding aorta, and right ventricular hypertrophy [2]. Severe cases of TOF often result in chronic hypoxemia and progressive right ventricular dysfunction, predisposing patients to heart failure and increased mortality risk [3].

Surgical correction remains the definitive treatment for TOF, with early intervention being crucial for improving long-term survival [4,5]. Studies have demonstrated that corrective surgery performed before the age of three years significantly reduced mortality and improved functional outcomes by alleviating right ventricular pressure overload, optimizing pulmonary blood flow, and preventing complications such as brain abscesses and stroke [1,6]. However, open-heart surgery involving cardiopulmonary bypass provokes a systemic inflammatory response syndrome (SIRS), which could lead to major organ dysfunction and death [7]. Prolonged systemic hyperinflammation induces immunosuppression and increases infection risk [8]. Additionally, aortic clamping during surgery promotes hypo-oncotic conditions, ischemia, and intestinal permeability, facilitating endotoxin translocation and exacerbating inflammation [6,8]. The intraoperative release of pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs) activates the NLRP3 inflammasome (NOD-, LRR-, and pyrin domain-containing protein 3 also known as NOD-like receptor protein 3), triggering the caspase-1 signaling cascade and the subsequent release of interleukin-1β (IL-1β) [9,10]. This inflammatory response is counterbalanced by an increase in interleukin-10 (IL-10), reflecting an anti-inflammatory mechanism aimed at modulating immune homeostasis [8]. Addressing perioperative inflammation is essential for optimizing recovery and improving long-term prognosis in TOF patients undergoing open-heart surgery.

The ketogenic diet, a high-fat, low-carbohydrate dietary approach, has been extensively studied for its metabolic effects and potential anti-inflammatory properties [11]. Modified Atkins diet (MAD), a ketogenic diet, offers several advantages, including ease of implementation in resource-limited settings, cost-effectiveness, feasibility for outpatient management, and overall practicality [12]. This dietary approach induces physiological ketosis by promoting the production of ketone bodies, which serve as alternative energy substrates and may contribute to cardioprotective effects through metabolic and biochemical adaptations [13-16]. Among these ketone bodies, β-hydroxybutyrate, a short-chain fatty acid, has protective effects on lung epithelial cells by reducing oxidative stress, plays a crucial role in suppressing cytokine release and prevents pyroptosis and apoptosis by directly targeting the NLRP3 inflammasome, thereby mitigating inflammatory responses associated with surgical stress [12,17,18]. Furthermore, β-hydroxybutyrate suppresses pro-inflammatory signaling by inhibiting caspase-1 and IL-1β while simultaneously enhancing anti-inflammatory mechanisms, as evidenced by increased IL-10 release [18,19].

Despite the growing interest in nutritional interventions for perioperative care, there is a significant knowledge gap regarding the impact of ketogenic diets in pediatric cardiac surgery. To date, no study has explored the effect of MAD on TOF patients undergoing open-heart surgery. The aim of this study was to assess the effect of MAD on the expression of NLRP3, caspase-1, IL-1β, and IL-10, in TOF patients undergoing open-heart surgery. It was hypothesized that MAD could attenuate perioperative inflammation in TOF patients by modulating NLRP3 inflammasome activation, downregulating caspase-1 and IL-1β while promoting IL-10 production, thereby mitigating excessive inflammation and improving surgical outcomes.

Methods

Study design and setting

A double-arm, randomized-controlled trial was conducted in Cardiac Intensive Care Unit, Integrated Cardiac Center, Dr. Cipto Mangunkusumo Hospital and Jakarta Heart Center Hospital, Jakarta, Indonesia. Main laboratory tests and measurements were performed at the

Integrated Laboratory for Diagnostic and Research Center (DIARC), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, and the Laboratory of Anatomical Pathology, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. The patients were divided into two groups: modified Atkins group and control group. Modified Atkins underwent the MAD intervention, consisting of a low-carbohydrate, high-fat diet with unrestricted fat and protein intake, implemented for at least 14 days prior to open-heart surgery. The control group followed the standard preoperative dietary regimen, which did not include carbohydrate restriction. Blood plasma and infundibulum heart tissues were collected from the patients of which NLRP3, caspase-1, IL-1β, and IL-10 were measured.

Sample size, sampling method, randomization, and allocation

A total of 45 patients diagnosed with TOF and scheduled for open-heart surgery correction were recruited for the study in the period of December 2023 until October 2024. The sample size was calculated using the hypothesis testing formula for two groups with unpaired numerical analysis [17]. With a combined standard deviation of 1.8, the required sample size per group was 21, which was increased by 10% to account for potential dropouts. After randomization, 23 patients were assigned to the treatment group receiving the MAD, while 22 were allocated to the control group. A consecutive sampling method was used, enrolling all eligible patients who met the inclusion criteria until the required sample size was achieved. Patients were recruited from the Integrated Cardiac Center, Dr. Cipto Mangunkusumo Hospital, and Jakarta Heart Center Hospital, Jakarta, Indonesia. Patients were randomly assigned to either the treatment or control group using block randomization with a block size of 4, ensuring a balanced distribution between the groups [20]. No blinding was implemented for either the researcher or the included patients.

Eligibility criteria

Patients diagnosed with TOF who underwent open-heart surgery correction were included in the study. Exclusion criteria included mortality due to death from unmanaged severe cyanotic attack in their home region. Dropout criteria were defined as non-adherence to the MAD protocol, specifically exceeding 30% carbohydrate intake.

Intervention

The MAD intervention consisted of a low-carbohydrate, high-fat diet with unrestricted fat and protein intake, implemented for at least 14 days prior to open-heart surgery correction. Carbohydrate intake was strictly limited to maintain ketosis, ensuring that total daily consumption did not exceed 30% of total energy intake. The daily protein requirement was set at 2 grams per kilogram of ideal body weight, ensuring adequate protein for growth, tissue repair, and metabolic demands without interfering with ketosis. Fat consumption was unrestricted, serving as the primary energy source to promote ketone production. The control group followed the standard preoperative dietary regimen, which did not include carbohydrate restriction. Patients in the control group consumed a balanced diet based on general nutritional guidelines for preoperative care, ensuring adequate caloric intake to maintain metabolic stability before surgery.

Sample collection

Whole blood samples were collected before and after intervention, as well as at 6-, 24-, and 48-hours post-surgery. In control group, the sample before the intervention was not collected due to ethical issue. Blood was centrifuged at 3500 rpm for 15 minutes, and 1 mL of plasma was stored at -20°C for Luminex analysis (Merck KGaA, Darmstadt, Germany) at the Integrated Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta. Additionally, infundibulum heart tissue samples were collected during surgery, fixed in 10% formalin, and processed for immunohistochemical examination at the Integrated Cardiac Center, Dr. Cipto Mangunkusumo, and Jakarta Heart Center Hospital, Jakarta.

NLRP3 and caspase-1 protein expression measurement

Antigen retrieval was conducted using heat-induced epitope retrieval (HIER) in tris-EDTA buffer (pH 9) at 96°C. The NLRP3 primary antibody (Polyclonal Antibody, GeneTex, California, USA)

was incubated overnight for 18 hours at a 1:300 dilution, and the caspase-1 antibody (Polyclonal Antibody, GeneTex, California, USA) was incubated for 2 hours at a 1:300 dilution. Secondary antibodies labeled with HRP (PVP250D antibody) were applied, and the slides were incubated before visualization using DAB chromogen solution. The slides were then examined under a light microscope.

NLRP3 and caspase-1 expression were evaluated by two independent pathologists. To prevent false-positive results due to connective tissue staining, expression was manually verified despite using software. All slides were scanned at 200× magnification using QuPath version 0.5.1 (https://qupath.github.io/). Positive expression of NLRP3 and caspase-1 was identified as brown cytoplasmic staining in cardiomyocytes, whereas unstained or blue-stained cells were considered negative. Staining intensity was scored as follows: strong brown (+3), medium brown (+2), brownish-yellow (+1), and no brown staining (0, negative expression). Scoring was based on five representative fields per slide at 200× magnification. NLRP3 and caspase-1 percentages were assessed using a Histoscore (H-score) at 200× magnification across 500 cells per sample. The H-score was calculated using the formula: H-score=(1×(% cells 1+)+2×(% cells 2+)+3×(% cells 3+)), yielding a range of 0–300. The optimal H-score cut-off was determined from a receiver operating characteristic (ROC) curve, with a significance set at $p<0.05$ [21,22].

IL-1β and IL-10 levels measurement

The concentrations of IL-1β and IL-10 were measured using a particle-based immunoassay with fluorescent-coded magnetic beads and Magplex-C microspheres (Luminex, Merck KGaA, Darmstadt, Germany) following the manufacture protocol. A total of 25 µL of sample was used and the results were read on the Luminex system (100 µL, 50 beads per bead set).

β-hydroxybutyrate colorimetric measurement

The remaining plasma specimens stored after the Luminex examination are removed from a temperature of -20°C for β-hydroxybutyrate level examination with colorimetric assay. The β Hydroxybutyrate Colorimetric Assay Kit (Abcam, Cambridge, UK) was used following the manufacturer's protocol. Potential interference from reduced pyridine nucleotides, such as NAD(P)H, was considered; if these compounds were suspected to be present in the sample, a background control was run to account for their effects. The reaction mix was used to quantify the amount of β-hydroxybutyrate in each sample. This assay kit is specific for β-hydroxybutyrate and does not cross-react with other ketones.

Statistical analysis

Statistical analysis was conducted using SPSS software version 24.0 (IBM, New York, USA), with $p<0.05$ considered statistically significant. Data normality was assessed using the Shapiro-Wilk test. For normally distributed data, the independent t-test was used for comparisons between groups, while for non-normally distributed data, the Mann-Whitney test and Wilcoxon signed-rank test were applied. The cut-off value and the predictive efficacy of NLRP3 and caspase-1 expression in the infundibulum heart tissue of TOF patients were assessed using the ROC curves.

Results

Patient recruitment and selection

A total of 45 patients were included in the recruitment process, with 23 patients receiving the MAD intervention, while 22 patients were allocated to the control group (**Figure 1**). Among the 23 patients in the treatment group, one patient was excluded due to meeting the dropout criteria (exceeding 30% carbohydrate intake). Seven out of 22 patients in the treatment group (one from the Dr. Cipto Mangunkusumo Hospital, and six from Jakarta Heart Center Hospital) had surgery schedules moved forward, preventing the administration of the MAD for 14 days before surgery. Following a discussion, these seven patients were given exogenous ketone supplementation capsules (3×1 dose for five days before surgery) and remained included in the study. No adverse events, such as diarrhea or vomiting, were reported. Among the 22 patients, 10 experienced surgical delays exceeding one month due to limited availability of oxygenators. The extended duration of the MAD due to surgical delays led to dietary fatigue in some patients, reducing

compliance. To address this, parents were engaged through Zoom meetings, WhatsApp photo monitoring of patient menus, and alternative MAD menu options, especially during the critical two-week pre-surgery period.

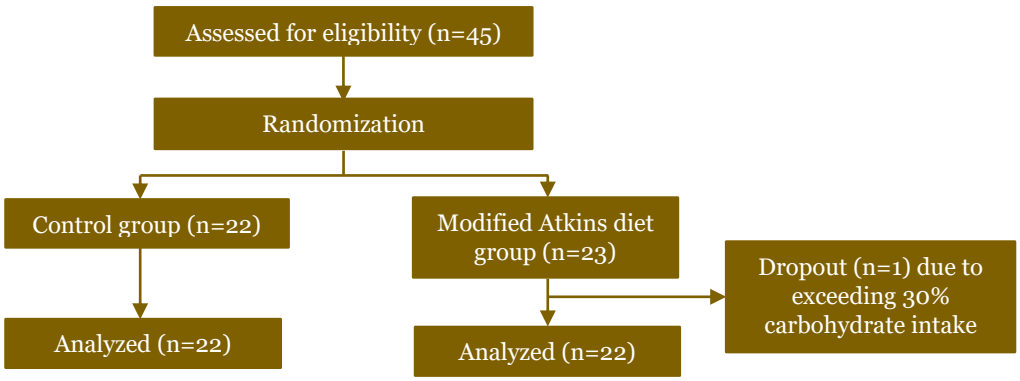

Figure 1. Simplified CONSORT flowchart of patient recruitment in this study.

Characteristics of the included TOF patients

This study analyzed 44 subjects, including 22 who received the MAD and 22 who received standard nutrition (control). Of the 22 subjects in the intervention group, 11 underwent open-heart surgery at the Integrated Cardiac Center, Dr. Cipto Mangunkusumo Hospital, and 11 at Jakarta Heart Hospital (**Table 1**). Geographically, 12 subjects were from Java, while 10 were from outside Java. The majority of the patients were male (n=13), with nine females. The mean age was 34 months, with a median of 26 months and an age range of 13–72 months. Two subjects had a cross-clamp time exceeding one hour, while 20 had a cross-clamp time of less than one hour (**Table 1**).

Table 1. Demographic and clinical characteristics between intervention and control groups

Variables	Modified Atkins diet (n=22)	Control (n=22)
	n (%)	n (%)
Age at operation (month), median (min-max)	26 (13–72)	26 (13–72)
Sex		
Male	13 (59%)	17 (77%)
Female	9 (41%)	5 (23%)
Nutritional status		
Good nutrition	18	15
Malnutrition	4	7
Length of modified Atkins diet program		
≤14 days	7	0
≥14 days	15	0
Cross clamp time		
≤1 hour	20	20
≥1 hour	2	2
Hospital		
Dr. Cipto Mangunkusumo	11	8
Jakarta Heart Center	11	14
Home region		
Java	12	13
Outside Java	10	9

Effect of modified Atkins diet (MAD) on NLRP3 and caspase-1 protein expression in tetralogy of Fallot (TOF) patients undergoing open-heart surgery

In the treatment group, NLRP3 protein expression varied from the absence of brown staining (-) to medium brown staining (+2) in the cardiomyocytes (**Figure 2A**), while caspase-1 protein expression showed the absence of brown staining (-) in the cytoplasm (**Figure 2B**). In contrast, the control group exhibited higher NLRP3 protein expression, ranging from brownish-yellow staining (+1) to strong brown staining (+3) (**Figure 2C**), and caspase-1 protein expression demonstrated brownish-yellow staining (+1) in the cardiomyocytes (**Figure 2D**). These findings

further confirmed that the MAD may have attenuated inflammasome activation, as evidenced by the reduction in NLRP3 and caspase-1 protein expression.

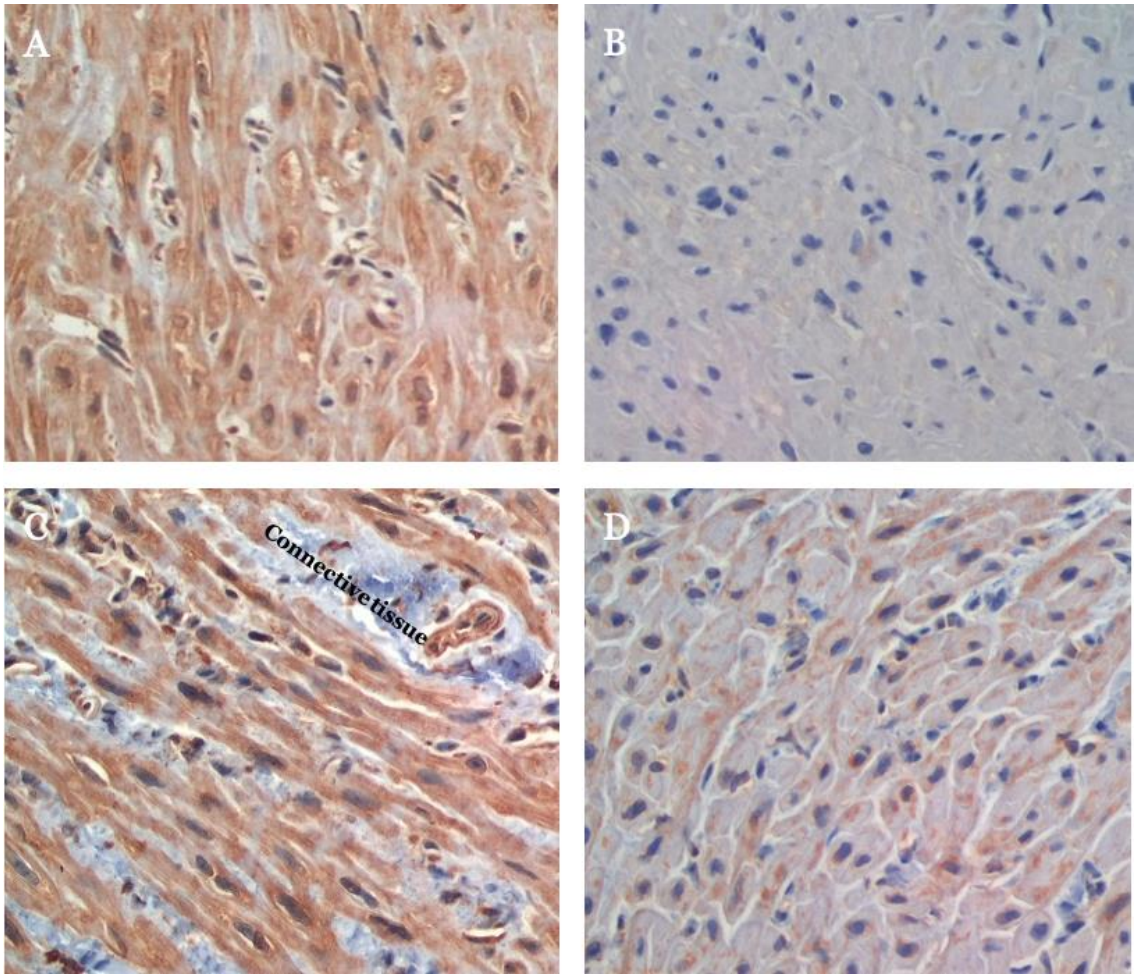

Figure 2. Immunohistochemistry analysis of NLRP3 and caspase-1 expression in the treatment and control groups on heart tissues of tetralogy of Fallot (TOF) patients undergoing open-heart surgery. (A) In the modified Atkins diet (MAD) group, NLRP3 expression was found with a range of negative to positive 2 (+2) and (B) negative expression of caspase-1 in the cytoplasm of the cardiomyocytes. (C) In the standard nutritional care group (control), NLRP3 expression was found with a range of positive 1 (+1) to positive 3 (+3) and (D) positive expression of 1 (+1) caspase-1 in the cytoplasm of the cardiomyocytes. The MAD group showed a decrease in NLRP3 and caspase-1 expression compared to standard nutritional nutrition.

NLRP3 protein expression was significantly lower in the treatment group, with a median H-score of 35.61 (0.50–117.69), compared to 58.59 (20.99–112.76) in the control group (p=0.015) (**Table 2** and **Figure 3**). Similarly, caspase-1 protein expression was significantly lower in the treatment group, with a median of 7.56 (0.66–62.75) compared to 31.68 (2.14–136.89) in the control group (p=0.001) (**Table 2** and **Figure 3**). These findings suggested that the MAD may play a role in modulating inflammatory pathways by suppressing NLRP3 inflammasome activation and reducing caspase-1 levels in TOF patients.

Table 2. Effect of modified Atkins diet (MAD) on NLRP3 and caspase-1 protein expression in infundibulum heart tissue of tetralogy of Fallot (TOF) patients undergoing open-heart surgery

Variable	Treatment	Control	p-value[a]
NLRP3 expression score, median (min-max)	35.61 (0.50–117.69)	58.59 (20.99–112.76)	0.015
Caspase-1 expression score, median (min-max)	7.56 (0.66–62.75)	31.68(2.14–136.89)	0.001

[a]Analyzed using Mann-Whitney U test

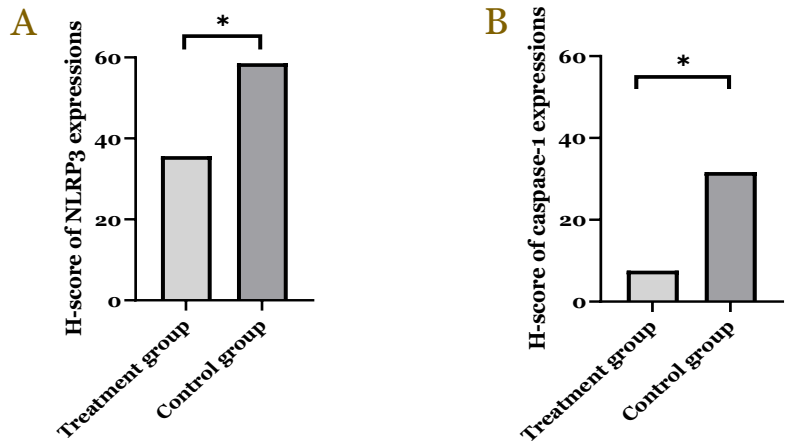

Figure 3. Effect of modified Atkins diet (MAD) on NLRP3 (A) and caspase-1 protein expression score (B) in tetralogy of Fallot (TOF) patients undergoing open-heart surgery. *Statistically significant at $p<0.05$.

Histopathological analysis of NLRP3 and caspase-1 expression was conducted, and semi-quantitative assessment was performed using the H-score formula across 500 cardiomyocytes. The results were categorized based on cut-off points from the ROC curve and Youden's index [21]. The ROC curve was generated to compare NLRP3 and caspase-1 expression between intervention and control groups, with the area under the curve (AUC) serving as a diagnostic strength indicator. Our data indicated that the area under the ROC curve of NLRP3 and caspase-1 was 0.715 (95%CI: 0.561–0.868), p=0.015 and 0.801 (95%CI: 0.669–0.932), p=0.001, respectively (**Figure 4**).

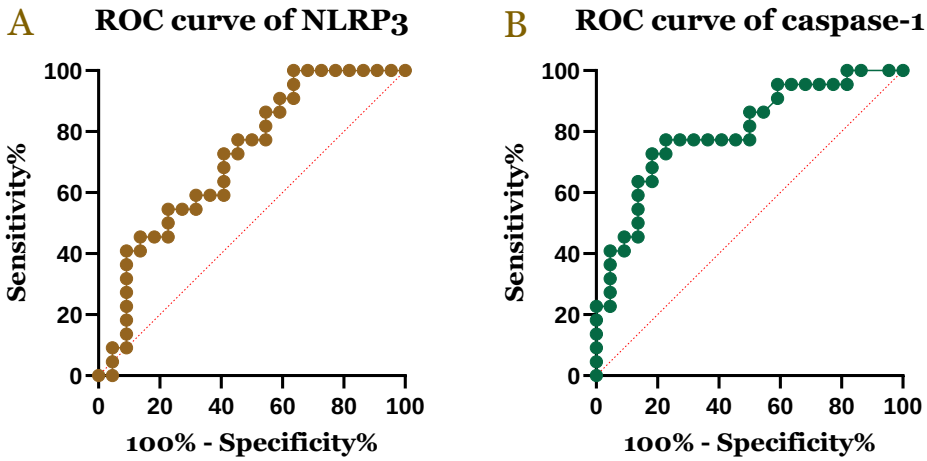

Figure 4. Receiver operating characteristic (ROC) curve of NLRP3 (A), and caspase-1 H-score (B) as diagnostic biomarkers. The cut-off between sensitivity and specificity of NLRP3 and caspase-1 was best characterized at 20.99 and 20.80, respectively.

The optimal cut-offs were calculated using Youden's index: (1) NLRP3: H-score ≥20.99 indicated a high likelihood of belonging to the control group and (2) caspase-1: H-score ≥20.80 suggested a strong likelihood of being in the control group. With an AUC of 0.801, caspase-1 demonstrated superior diagnostic value compared to NLRP3. Based on ROC-derived cut-off points, it showed that NLRP3 has a cut-off of 20.99 with 100% (correctly identifying all control cases) but low specificity (36.4%), making it less effective at distinguishing treatment cases. The positive predictive value (PPV) and negative predictive value (NPV) were 61.1% and 100%, respectively. Caspase-1, with a cut-off value of 20.8, demonstrated 72.7% sensitivity and 81.8% specificity, enabling more accurate identification of the treatment group than NLRP3. The PPV and NPV were 80.0% and 75.0%, respectively.

Effect of modified Atkins diet (MAD) on IL-1β and IL-10 levels in tetralogy of Fallot (TOF) patients undergoing open-heart surgery

In the treatment group, IL-1β levels decreased from before intervention to 48 hours post-surgery (**Figure 5**). Conversely, in the control group, IL-1β levels increased following the control diet, peaking at 6 hours post-surgery before subsequently declining at 24- and 48-hours post-surgery (**Figure 5**).

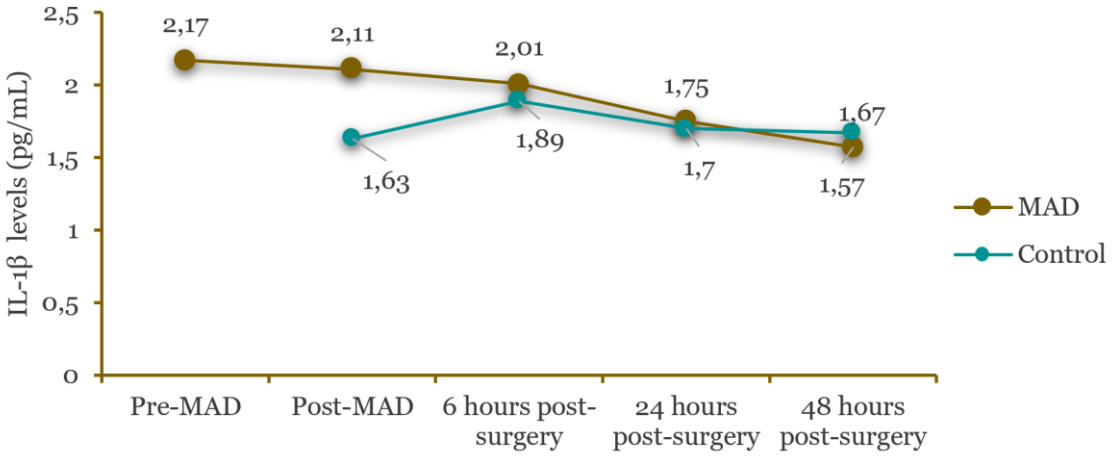

Figure 5. Comparison of interleukin-1β (IL-1β) level changes in modified Atkins diet (MAD) and control group before and after intervention, as well as at 6-, 24-, and 48-hours post-surgery in tetralogy of Fallot (TOF) patients undergoing open-heart surgery.

The treatment group was intended to follow the MAD for at least 14 days before surgery. However, variations in surgical schedules led to differences in adherence duration. Some patients experienced delays in their surgery, extending their diet period beyond 14 days, while others had their surgery scheduled earlier to fill vacant slots, making it impossible to complete the full 14-day diet. To mitigate this, patients who underwent surgery earlier received ketone capsules (3×1) for five days to maintain metabolic adaptation. Despite these adjustments, no statistically significant difference was observed in IL-1β levels before and after the diet intervention (p=0.357) (**Table 3**).

IL-1β levels showed no significant differences between post-diet and 6 hours post-surgery (p=0.709), post-diet and 24 hours post-surgery (p=0.322), or post-diet and 48 hours post-surgery (p=0.135). Similarly, in the control group, IL-1β levels did not significantly differ between post-standard preoperative nutritional diet and 6 hours post-surgery (p=0.940) or 24 hours post-surgery (p=0.961), with no significant difference observed at 48 hours post-surgery (p=0.168). Furthermore, the Mann-Whitney U test indicated no statistically significant difference in IL-1β levels between patients following the MAD and those on the standard preoperative nutritional diet (p=0.372) (**Table 3**).

In the treatment group, IL-10 levels increased from baseline following the dietary intervention, reaching a peak at 6 hours post-surgery (median: 40.73 pg/mL) before declining at 24- and 48-hours post-surgery (**Figure 6**). A similar trend was observed in the control group, where IL-10 levels increased before surgery, peaked at 6 hours post-surgery (median: 47.07 pg/mL), and subsequently declined at 24- and 48-hours post-surgery (**Figure 6**).

In the treatment group, no significant difference was observed in IL-10 levels before and after 14 days of the MAD intervention (p=0.931) (**Table 3**). However, IL-10 levels demonstrated significant differences between after MAD and 6 hours post-surgery (p=0.001), after MAD and 24 hours post-surgery (p=0.003), as well as after MAD and 48 hours post-surgery (p=0.024) (**Table 3**). Similarly, in the control group, significant differences in IL-10 levels were observed between after standard preoperative nutritional diet and 6 hours post-surgery (p=0.004) and between after standard preoperative nutritional diet and 24 hours post-surgery (p=0.009); however, no significant difference was found between after standard preoperative nutritional diet and 48 hours post-surgery (p=0.654) (**Table 3**). Furthermore, the Mann-Whitney test revealed

no significant difference in IL-10 levels between patients who received MAD and those who underwent standard preoperative nutritional diet (p=0.579) (**Table 3**).

Table 3. Effect of modified Atkins diet (MAD) on interleukin-1β (IL-1β) and interleukin-10 (IL-10) levels in tetralogy of Fallot (TOF) patients undergoing open-heart surgery

Variable	Treatment group		Control group		p-value[b]
	Median (min-max)	p-value[a]	Median (min-max)	p-value	
IL-1β (pg/mL)					0.372
Before intervention	2.17 (0.76–14.39)	0.357[1]	NA	NA	
14 days of intervention	2.11 (0.36–17.88)	Ref	1.63 (0.77–587.78)		
6 h post-surgery	2.01 (0.36–14.11)	0.709[2]	1.89 (0.95–23.06)	0.940[a2]	
24 h post-surgery	1.75 (0.87–15.26)	0.322[3]	1.70 (0.77–9.80)	0.961[a3]	
48 h post-surgery	1.57 (0.83–14.31)	0.135[4]	1.67 (0.30–7.73)	0.168[a4]	
IL-10 (pg/mL)					0.579
Before intervention	6.95 (1.34–84.49)	0.931[1]	NA	NA	
14 days of intervention	9.31 (1.97–69.08)	Ref	6.40 (1.47–846.68)		
6 h post-surgery	40.73 (1.97–614.06)	0.001[2*]	47.07 (3.23–1964.75)	0.004[a2*]	
24 h post-surgery	16.27 (4.13–1240.62)	0.003[3*]	18.30 (2.90–1539.49)	0.009[a3*]	
48 h post-surgery	8.18 (2.33–1048.03)	0.024[4*]	7.50 (1.97–2829.21)	0.654[a4]	

NA: not available, sample in control group was not collected due to ethical issue
[a]Analyzed using Wilcoxon signed-rank test
[b]Analyzed using Mann-Whitney U test, comparison of IL-1β and IL-10 levels between MAD and control group (for all time points)
[1–4]Represented statistical comparisons: 1 for before vs after intervention, 2 for after intervention vs 6 hours post-surgery, 3 for after intervention vs 24 hours post-surgery, and 4 for after intervention vs 48 hours post-surgery
[*]Statistically significant at p<0.05

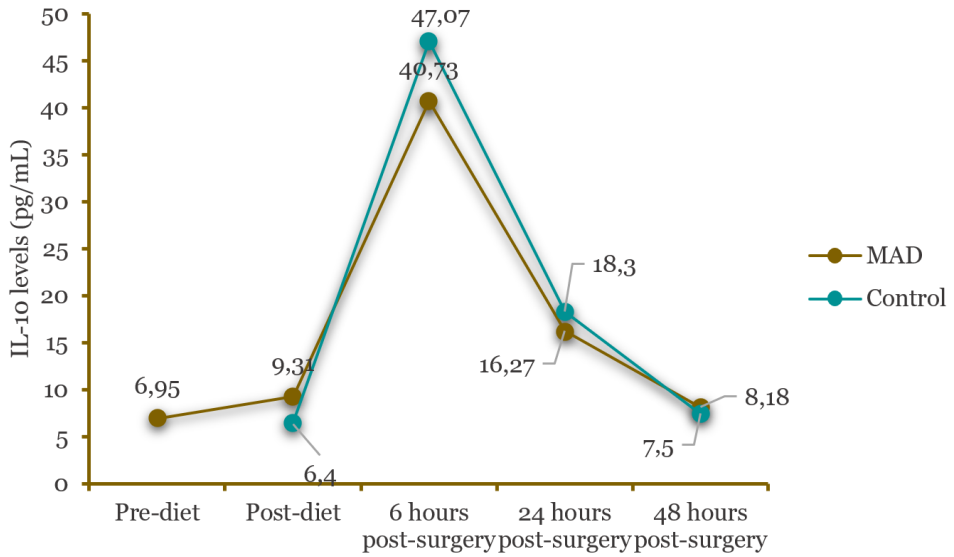

Figure 6. Comparison of interleukin-10 (IL-10) level changes in modified Atkins diet (MAD) and control group before and after intervention, as well as at 6-, 24-, and 48-hours post-surgery in tetralogy of Fallot (TOF) patients undergoing open-heart surgery.

Effect of modified Atkins diet (MAD) on β-hydroxybutyrate enzyme levels in tetralogy of Fallot (TOF) patients undergoing open-heart surgery

The effect of MAD on β-hydroxybutyrate enzyme level was measured after the treatment was completed. The highest plasma β-hydroxybutyrate level in the treatment group was 2.19 mmol/L, observed in case number 10 after 29 days of the MAD, whereas the lowest level was 0.16 mmol/L after 7 days. In the control group, the highest and lowest plasma β-hydroxybutyrate levels were 0.74 mmol/L and 0.12 mmol/L, respectively (**Figure 7**). The median plasma β-hydroxybutyrate level was similar between the treatment group (0.26 mmol/L with ranges of 0.16–2.19) and control group (0.26 mmol/L with ranges of 0.12–0.74), p=0.411.

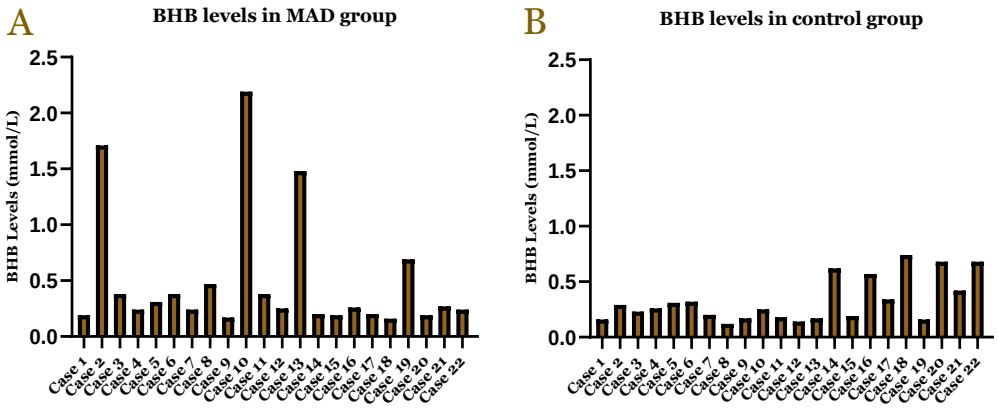

Figure 7. Comparison of β-hydroxybutyrate levels between the modified Atkins diet (MAD) group (A) and control group (B) in tetralogy of Fallot (TOF) patients undergoing open-heart surgery.

Discussion

To the best of our knowledge, this study is the first to explore NLRP3 and caspase-1 protein expression in cardiomyocytes from the infundibular tissues of children with TOF, whereas previous report was limited to animal models [23]. NLRP3 inflammasome and caspase-1 have been reported to enhance reactive oxygen species (ROS) production and IL-1β levels in cardiac cells of mice subjected to a diet high in refined carbohydrates [24]. In this study, NLRP3 protein expression was significantly lower in the MAD group than in the control group (p=0.015), and caspase-1 protein expression was also significantly reduced in the MAD (p=0.001), suggesting that MAD may play a role in modulating inflammatory pathways by suppressing NLRP3 inflammasome activation and reducing caspase-1 protein expression in TOF patients undergoing open-heart surgery [12,17]. The AUC for NLRP3 and caspase-1 indicate that these biomarkers had the potential to be an inflammatory diagnostic biomarker, with caspase-1 demonstrating a better diagnostic potential compared to NLRP3.

These findings were aligned with previous reports indicating that ketogenic diets influence NLRP3 protein expression and its signaling pathways [17,19,25]. Reduced expression of NLRP3 and caspase-1 may exert a protective effect against hyperinflammation induced by ischemia-reperfusion injury during cardiac surgery utilizing cardiopulmonary bypass [10]. Therefore, the MAD may serve as a potential nutritional intervention to mitigate inflammatory responses in patients with TOF undergoing open-heart surgery, potentially reducing the risk of hyperinflammation associated with ischemia-reperfusion injury during cardiopulmonary bypass [8,12].

This study did not identify significant changes in IL-1β and IL-10 levels before and after the treatment in either the MAD or control groups. This may be attributed to IL-10 being primarily produced during active inflammation to restore homeostasis. However, in the treatment group, IL-10 levels significantly decreased at 48 hours post-surgery, returning closer to preoperative levels compared to the control group, potentially reflecting reduced hyperinflammatory responses associated with lower NLRP3 and caspase-1 expression [26,27]. The low expression of NLRP3 and caspase-1, alongside stable IL-1β levels, may be attributed to regulatory mechanisms of inflammasome activation and IL-1β processing. IL-1β is initially synthesized as an inactive precursor (pro-IL-1β) and requires cleavage by caspase-1 for activation [28]. Reduced NLRP3 and caspase-1 protein expression in this study suggested limited inflammasome activation, thereby decreasing pro-IL-1β processing. However, IL-1β may remain unchanged if it is already present in its active form or generated through alternative pathways, such as non-canonical inflammasome activation or other proteases [29,30].

NLRP3 inflammasome activation is typically triggered by pathogenic or danger signals, leading to caspase-1 activation and subsequent cleavage of pro-IL-1β [10]. However, IL-1β can also be generated independently of NLRP3 and caspase-1 through alternative inflammatory pathways [31,32]. The low expression of NLRP3 and caspase-1 observed in this study suggests limited inflammasome activation, reducing pro-IL-1β processing. If active IL-1β remains at

normal levels despite suppressed NLRP3 and caspase-1 expression, it may result from pre-existing IL-1β or alternative processing mechanisms, such as caspase-8 or other inflammatory proteases [33,34]. Various factors influence IL-1β levels, including the surrounding inflammatory environment, where strong cytokine signals can sustain IL-1β production independently of NLRP3 or caspase-1 activity [35]. Additionally, regulatory feedback mechanisms may stabilize or enhance IL-1β secretion even in the absence of full inflammasome activation [36]. While NLRP3 and caspase-1 are key mediators of IL-1β activation, alternative pathways, pre-existing active IL-1β, or compensatory mechanisms within the cellular environment may maintain IL-1β levels [37,38].

This study demonstrated a transient increase in IL-10 levels at 6 hours post-surgery in the MAD group, followed by a gradual decline at 24 and 48 hours. In contrast, the control group did not exhibit a significant reduction in IL-10 levels over the same period. The sustained presence of IL-10 in the treatment group suggested a prolonged anti-inflammatory response, which may have contributed to better immune regulation during the postoperative period. Given IL-10's role in counteracting excessive inflammation, its persistence up to 48 hours post-surgery indicates that the metabolic effects of ketosis or sub-ketosis induced by the MAD may have facilitated a more effective resolution of systemic inflammation [25]. This suggests a potential benefit in maintaining perioperative metabolic modulation to improve immune homeostasis following surgical stress [26,27].

PAMPs, DAMPs, IL-1β, and tumor necrosis factor-alpha (TNF-α) signaling, which induce the MYD88 adapter protein, leading to phosphorylation of interleukin-1 receptor-associated kinase (IRAK) and TNF receptor-associated factor (TRAF) [39]. This process triggers phosphorylation and degradation of inhibitor of κB (IκB), allowing p65/p50 nuclear factor kappa-B (NF-κB) dimer formation and subsequent activation of the NF-κB pathway [10,39]. This promotes the transcription of NLRP3, pro-IL-1β, and pro-IL-18. During activation, the NLRP3 receptor recruits apoptosis-associated speck-like protein containing a caspase recruitment domain, which interacts with pro-caspase-1 to form the inflammasome complex [10]. NIMA-related kinase 7 (NEK7) binding facilitates inflammasome oligomerization. Potassium efflux, sodium and calcium influx, ROS production, mitochondrial DNA release, and the presence of oxidized low-density lipoprotein (oxLDL) or cholesterol crystals contribute to inflammasome assembly by inducing lysosomal damage [10]. Chloride efflux further promotes NEK7 binding and oligomerization. Caspase-1 undergoes autoproteolysis at the inflammasome complex, cleaving pro-IL-1β and pro-IL-18 into their mature, active forms, IL-1β and IL-18 [10].

This study confirmed that β-hydroxybutyrate inhibits the NLRP3 receptor. Specifically, β-hydroxybutyrate suppressed the pyrin domain (PYD) of the N-terminal region of NLRP3 and the central region of caspase-1, leading to a significant reduction in NLRP3 and caspase-1 expression in the treatment group compared to the control group [36]. However, it remains unclear whether β-hydroxybutyrate directly suppresses IL-1β, as no significant differences were observed in IL-1β levels between before and after MAD, after MAD and 6 hours post-surgery, after MAD and 24 hours post-surgery, or after MAD and 48 hours post-surgery. Despite the reduction in NLRP3, and caspase-1 protein expression, IL-1β levels remained stable, and the inflammatory response persisted, as indicated by a significant increase in IL-10 following surgery [40,41].

IL-1β production can be mediated through alternative pathways, including non-canonical inflammasome activation, caspase-1-independent mechanisms, cytokine-induced pathways, cellular stress responses, microbial products, and inflammatory feedback loops [31]. These pathways often involve post-translational modifications or regulatory mechanisms that stabilize IL-1β secretion despite reduced NLRP3 and caspase-1 protein expression [29,30]. IL-10 production was induced by pro-inflammatory cytokines, reaching peak levels at 6 hours post-surgery before declining at 24 and 48 hours, signifying the resolution of inflammation following open-heart surgical correction[10,25]. Upon binding to its receptor, IL-10 activates the JAK and TYK-2 pathways, leading to the phosphorylation of signal transducer and activator of transcription 3 (STAT3) [27]. Phosphorylated STAT3 forms a homodimer and undergoes nuclear translocation where it binds to STAT3-responsive elements, promoting the gene expression of anti-inflammatory mediators that suppress inflammatory signaling [27]. IL-10-responsive gene products inhibit Toll-like receptor signaling, interleukin receptor pathways, and key adaptor

proteins such as MYD88, IRAK, and TRAF6 [27]. This suppression prevents the activation of transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 2/3 (TAB2/3) and NF-κB, thereby downregulating TNF-α and IL-1β expression, while also modulating IL-6 signaling through JAK3 pathways [26,27].

However, the release of IL-10 is not exclusively triggered by IL-1β secretion [27]. Antigen-presenting cells, such as dendritic cells, can induce IL-10 expression in response to pathogen recognition, while cytokines such as IL-21 (produced by T-helper 1 cells), and IL-27 (produced by T-helper 1, T-helper 2, T-helper 17, and regulatory T cells) enhance its production through STAT1- and STAT3-dependent mechanisms [32]. Additionally, IL-6, and TNF-α activate the IRAK, TRAF6, TAK1, and NF-κB signaling pathways, further promoting IL-10 expression via STAT3 activation [42].

This study did not measure IL-18, another cytokine released through NLRP3 inflammasome activation, nor the cytokines TNF-α and IL-6, both of which can influence IL-10 secretion [26]. Increased IL-10 levels may also result from other inflammatory mediators, such as C-reactive protein (CRP), released by cells or tissues. Failure to restore homeostasis may exacerbate hyperinflammation [43,44]. Furthermore, hyperinflammation and SIRS are closely related, as both involve excessive immune activation [39,40]. Hyperinflammation is characterized by the uncontrolled release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [45], which can lead to tissue damage, cytokine storms, and multiple organ dysfunction syndrome [46,47]. MAD could serve as a valuable adjunct to conventional perioperative management in TOF patients, potentially reducing morbidity and improving recovery following open-heart surgery.

A common concern regarding the MAD is the potential for weight loss, which could adversely impact growth. However, this study did not observe such an effect. Notably, 21 out of 22 patients in the treatment group showed weight gain, with no cases of weight loss. This outcome may be attributed to the high-calorie intake provided to the intervention group, which ensured adequate protein and fat consumption without caloric restriction, despite maintaining very low carbohydrate levels [25].

Metabolic therapy, particularly dietary interventions such as MAD [12], has potential clinical applications in improving outcomes for patients with TOF and possibly other CHDs requiring open-heart surgery. Given the substantial inflammatory response and metabolic stress induced by cardiopulmonary bypass [8], targeted metabolic modulation may enhance prognosis, improve long-term survival, and optimize postoperative recovery [17]. Additionally, MAD may provide cardioprotective effects by improving mitochondrial function and reducing oxidative stress, potentially mitigating postoperative cardiac dysfunction and low cardiac output syndrome [13,14,15,16].

The optimal duration of MAD in TOF patients undergoing open-heart surgery remains unclear. While short-term interventions of 14 days may be sufficient to modulate perioperative inflammation, as presented in this study, extending therapy beyond 14 days may offer additional benefits, including prolonged metabolic adaptation, sustained anti-inflammatory effects, and improved immune homeostasis [10]. However, prolonged therapy must be carefully monitored to avoid potential metabolic imbalances or unintended consequences, such as excessive immunosuppression or altered metabolic adaptation that may impact growth and development in pediatric patients [12].

Given that hyperinflammation and metabolic stress are common across various CHD requiring cardiopulmonary bypass, metabolic therapy may also be applicable to conditions such as transposition of the great arteries following arterial switch operation, hypoplastic left heart syndrome undergoing staged palliation, total anomalous pulmonary venous return following surgical correction, and atrioventricular septal defect repair, particularly in syndromic cases with metabolic abnormalities [48,49,50]. However, further studies are required to determine the optimal duration, patient selection criteria, and long-term safety of metabolic interventions in this population [51,52].

Conclusion

MAD may modulate perioperative inflammation in TOF patients undergoing open-heart surgery by downregulating NLRP3 and caspase-1 expression while sustaining IL-10 levels. The sustained

IL-10 response at 48 hours post-surgery in the MAD group, compared to the transient response in the standard diet group, indicates a prolonged anti-inflammatory effect, potentially facilitating immune homeostasis. The unchanged IL-1β levels despite reduced NLRP3 and caspase-1 expression suggest alternative pathways contributing to IL-1β regulation. This study emphasizes the complexity of inflammatory responses in surgical stress and highlights the need for further investigation into the MAD's mechanistic role and long-term clinical impact on postoperative recovery and outcomes in congenital heart surgery.

Ethics approval

Protocol of this study was reviewed and approved by Ethical Committee for Health Research, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia (Approval number: 23-08-1311).

Acknowledgments

Gratitude is extended to the professional staffs and laboratories at Dr. Cipto Mangunkusumo Hospital, including the Anatomical Pathology Laboratory, the Integrated Laboratory, Faculty of Medicine, Universitas Indonesia; the Child Health Kiara Laboratory, Dr. Cipto Mangunkusumo Hospital; Jakarta Heart Center Hospital; Prodia Laboratory; and Biogen Laboratory. Sincere gratitude is expressed to all patients who participated in this study across Indonesia.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This research was partially funded by the International Publication Grant from Universitas Indonesia (Hibah Publikasi Terindeks Internasional (PUTI) Pascasarjana) 2024 (Grant number: NKB-77/UN2.RST/HKP.05.00/2024).

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities of which AI-based language models ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

How to cite

Yanuarso PB, Djer MM, Hendarto A, *et al*. Effect of the modified Atkins diet on NLRP3, caspase-1, IL-1β, and IL-10 in patients with tetralogy of Fallot undergoing open-heart surgery: A randomized controlled trial. Narra J 2025; 5 (1): e2138 - http://doi.org/10.52225/narra.v5i1.2138.

References

1. Kementerian Kesehatan Republik Indonesia. Kurangi kematian penyakit jantung bawaan pada bayi baru lahir dengan Cathlab. Available from: https://kemkes.go.id/id/%20kurangi-kematian-penyakit-jantung-bawaan-pada-bayi-baru-lahir-dengan-cathlab. Accessed: 15 October 2024.
2. Prasodo AM, Sukardi R. Cyanotic congenital heart disease. In: Sastroasmoro S, Djer MM, editors. Textbook of pediatric cardiology. Vol X. 2th edition. Jakarta: Indonesian Pediatric Society Publishing House; 2020.
3. Mandalenakis Z, Rosengren A, Skoglund K, *et al*. Survivorship in children and young adults with congenital heart disease in Sweden. JAMA Intern Med 2017;177(2):224-230.

4. van der Ven JPG, van den Bosch E, Bogers AJCC, Helbing WA. Current outcomes and treatment of tetralogy of Fallot. F1000Res 2019;8(F1000 Faculty Rev):1530.
5. Romeo JLR, Etnel JRG, Takkenberg JJM, *et al.* Outcome after surgical repair of tetralogy of Fallot: A systematic review and meta-analysis. J Thorac Cardiovasc Surg 2020;159(1):220-236.e8.
6. Sarkar M, Prabhu V. Basics of cardiopulmonary bypass. Indian J Anaesth 2017;61(9):760-767.
7. Squiccimarro E, Stasi A, Lorusso R, Paparella D. Narrative review of the systemic inflammatory reaction to cardiac surgery and cardiopulmonary bypass. Artif Organs 2022;46(4):568-577.
8. Justus G, Walker C, Rosenthal LM, *et al.* Immunodepression after CPB: Cytokine dynamics and clinics after pediatric cardiac surgery – A prospective trial. Cytokine 2019;122:154018.
9. Napodano C, Carnazzo V, Basile V, *et al.* NLRP3 inflammasome involvement in heart, liver, and lung diseases – A lesson from cytokine storm syndrome. Int J Mol Sci 2023;24(23):16556.
10. Cosentino N, Ashraf Z, Li F, *et al.* NLRPP inflammasome: The rising star in cardiovascular diseases. Front Cardiovasc Med 2022;9:927061.
11. Pinto A, Bonucci A, Maggi E, *et al.* Anti-oxidant and anti-inflammatory activity of ketogenic diet: New perspectives for neuroprotection in Alzheimer's disease. Antioxidants 2018;7(5):63.
12. Rafli A, Handryastuti S, Karyanti MR, *et al.* The effectiveness of modified Atkins ketogenic diet on children with intractable epilepsy: A pilot study from Indonesia. J Nutr Metab 2023;2023:9222632.
13. Kolb H, Kempf K, Röhling M, *et al.* Ketone bodies: From enemy to friend and guardian angel. BMC Med 2021;19(1):313.
14. White H, Heffernan AJ, Worrall S, *et al.* A systematic review of intravenous β-hydroxybutyrate use in humans – A promising future therapy?. Front Med 2021;8:740374.
15. Luong TV, Abild CB, Bangshaab M, *et al.* Ketogenic diet and cardiac substrate metabolism. Nutrients 2022;14(7):1322.
16. Liu M, Lv J, Pan Z, *et al.* Mitochondrial dysfunction in heart failure and its therapeutic implications. Front Cardiovasc Med 2022;9:945142.
17. Rahmel T, Hübner M, Koos B, *et al.* Impact of carbohydrate-reduced nutrition in septic patients on ICU: Study protocol for a prospective randomised controlled trial. BMJ Open 2020;10(7):e038532.
18. Mohammadifard N, Haghighatdoost F, Rahimlou M, *et al.* The effect of ketogenic diet on shared risk factors of cardiovascular disease and cancer. Nutrients 2022;14(17):3499.
19. Neal E. Alternative ketogenic diet. In: Masino SA, editor. Ketogenic diet and metabolic therapies: Expanded roles in health and disease. 2 edn. Oxford: Oxford University Press; 2022.
20. Kim J, Shin W. How to do random allocation (randomization). Clin Orthop Surg 2014;6(1):103-109.
21. Rachmadi L, Linggodigdo M, Hasan F, *et al.* Exploring the relationship between YAP (yes-associated protein) and VEGF-A with distant metastasis in nasopharyngeal carcinoma. Asian Pacific J Cancer Prev 2024;25(10):3507-3513.
22. Rachmadi L, Laelasari E, Susanto YDB, Kusmardi K. MMP-9 and CCR7 as possible predictors of lymph node metastasis in laryngeal squamous cell carcinoma. Iran J Pathol 2023;18(2):164-172.
23. Liu XC, Huang M, Huang XP, *et al.* NLRP3 is a promising target for regulating high glucose-induced inflammatory response in *Megalobrama amblycephala*. Aquaculture 2022;555:738220.
24. Castor RGM, Bruno AS, Pereira CA, *et al.* Glibenclamide reverses cardiac damage and NLRP3 inflammasome activation associated with a high refined sugar diet. Eur J Pharmacol 2024;(984):177035.
25. Noakes TD, Murphy T, Wellington N, *et al.* Kategonic: The science of therapeutic carbohydrate restriction in human health. Amsterdam: Academic Press; 2023.
26. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol 2010;10(3):170-181.
27. Gloria Kang GJ, Ewing-Nelson SR, Mackey L, *et al.* 乳鼠心肌提取 HHS public access. Physiol Behav 2018;176(1):139-148.
28. Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. Cell Discov 2020;6(1):36.
29. O'Keefe ME, Dubyak GR, Abbott DW. Post-translational control of NLRP3 inflammasome signaling. J Biol Chem 2024;300(6):107386.
30. Zhou YR, Dang JJ, Yang QC, Sun ZJ. The regulation of pyroptosis by post-translational modifications: Molecular mechanisms and therapeutic targets. EBioMedicine 2024;109:105420.
31. Pyrillou K, Burzynski LC, Clarke MCH. Alternative pathways of IL-1 activation, and its role in health and disease. Front Immunol 2020;11:613170.

32. Wan CK, Li P, Spolski R, *et al*. IL-21-mediated non-canonical pathway for IL-1β production in conventional dendritic cells. Nat Commun 2015;6:7988.
33. Chen ZH, Jin SH, Wang MY, *et al*. Enhanced NLRP3, caspase-1, and IL- 1β levels in degenerate human intervertebral disc and their association with the grades of disc degeneration. Anat Rec 2015;298(4):720-726.
34. Accogli T, Hibos C, Vegran F. Canonical and non-canonical functions of NLRP3. J Adv Res 2023;53:137-151.
35. Gurung P, Kanneganti TD. Novel roles for caspase-8 in IL-1β and inflammasome regulation. Am J Pathol 2015;185(1):17-25.
36. Swanson KV, Deng M, Ting JPY. The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. Nat Rev Immunol 2019;19(8):477-489.
37. Mao K, Chen S, Chen M, *et al*. Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. Cell Res 2013;23(2):201-212.
38. Ramachandran R, Manan A, Kim J, Choi S. NLRP3 inflammasome: A key player in the pathogenesis of life-style disorders. Exp Mol Med 2024;56(7):1488-1500.
39. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.
40. Warren OJ, Watret AL, de Wit KL, *et al*. The inflammatory response to cardiopulmonary bypass: Part 2-anti-inflammatory therapeutic strategies. J Cardiothorac Vasc Anesth 2009;23(3):384-393.
41. Warren OJ, Smith AJ, Alexiou C, *et al*. The inflammatory response to cardiopulmonary bypass: Part 1-mechanisms of pathogenesis. J Cardiothorac Vasc Anesth 2009;23(2):223-231.
42. Bailey J, Oliveri A, Levin E. IL-27 and IL-21 are associated with T cell IL-10 responses in human visceral leishmaniasis. J immunol 2011;186(7):3977-3985.
43. Tegeler C, O'Sullivan JL, Bucholtz N, *et al*. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function-data from the Berlin Aging Study II. Neurobiol Aging 2016;38:112-117.
44. Hassan J, Khan S, Zahra R, *et al*. Role of procalcitonin and C-reactive protein as predictors of sepsis and in managing sepsis in postoperative patients: A systematic review. Cureus 2022;14(11):e31067.
45. Sikora JP, Karawani J, Sobczak J. Neutrophils and the systemic inflammatory response syndrome (SIRS). Int J Mol Sci 2023;24(17):13469.
46. Comstedt P, Storgaard M, Lassen AT. The systemic inflammatory response syndrome (SIRS) in acutely hospitalised medical patients: A cohort study. Scand J Trauma Resusc Emerg Med 2009;17(1):67.
47. Robertson CM, Coopersmith CM. The systemic inflammatory response syndrome. Microbes Infect 2006;8(5):1382-1389.
48. Williams J, Stoll VM, Arif S, *et al*. Follow up after atrial switch surgery in patients with transposition of the great arteries; Reality versus guidelines. Int J Cardiol Congenit Heart Dis 2021;3:100123.
49. Czosek RJ, Anderson JB, Heaton PC, *et al*. Staged palliation of hypoplastic left heart syndrome: Trends in mortality, cost, and length of stay using a national database from 2000 through 2009. Am J Cardiol 2013;111(12):1792-1799.
50. Rao PS, Harris AD. Recent advances in managing septal defects: Ventricular septal defects and atrioventricular septal defects. F1000Res 2018;7(F1000 Faculty Rev):498.
51. Heibel J, Graham EM, Mahle WT, *et al*. Perioperative metabolites are associated with adverse neonatal congenital heart disease surgical outcomes. J Am Heart Assoc 2022;11(16):e024996.
52. Meggiolaro L, Moschino L, Stocchero M, *et al*. Metabolomic profiling of infants undergoing cardiopulmonary bypass and association with clinical outcomes: A systematic review. Front Cardiovasc Med 2024;11:1491046.