Heart Surgery Forum 2024; 27(2): E119–E125
doi: 10.59958/hsf.6997
https://journal.hsforum.com/

Article

Oxidative Stress after on-Pump Cardiac Surgery in Patients with Preoperative Renal Dysfunction

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Submitted: 15 November 2023 Revised: 13 December 2023 Accepted: 27 December 2023 Published: 19 February 2024

Abstract

Background: Renal dysfunction is a global health burden with a rising prevalence and increased morbidity and mortality. Since the complex and multifactorial pathophysiology of this disease and its consequences is not entirely understood, novel mechanisms are currently under investigation, one being oxidative stress. Malondialdehyde is a product of lipid peroxidation and has been widely utilised as an indirect biomarker of oxidative stress. The aim of this study was to investigate the perioperative oxidative stress in patients with preoperative renal dysfunction undergoing on-pump cardiac surgery. **Methods**: In 115 patients scheduled for on-pump cardiac surgery, serum concentrations of malondialdehyde were obtained pre-, intra- and postoperatively. The patients were enrolled into two study groups regarding their preoperative renal function, and the malondialdehyde concentrations were compared between the study groups. **Results**: Patients with preoperative renal dysfunction were older and had a higher mean EuroSCORE II score. On postoperative days 1, 2 and 3, the serum malondialdehyde levels were significantly higher in patients with preoperative renal dysfunction compared to those with normal kidney function. Using regression analysis, preoperative renal dysfunction was shown to be an independent predictor of higher postoperative malondialdehyde levels at all tested time points. **Conclusions**: In a pioneering study correlating cardiopulmonary bypass and oxidative stress biomarker malondialdehyde, patients with preoperative renal dysfunction were found to exhibit more pronounced and prolonged oxidative stress resulting in protracted lipid peroxidation in the early postoperative period compared to patients with normal kidney function.

Keywords

renal dysfunction; lipid peroxidation; malondialdehyde; cardiopulmonary bypass

Introduction

Preoperative renal dysfunction with an estimated glomerular filtration (eGFR) of equal or less than 89 mL/min/1.73 m^2 as recommended by the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) [1] is associated with increased morbidity and mortality after major surgery [2]. The reported annual mortality in these patients is up to 20-fold higher than in the general population [3], and it carries a 5- to 10-fold higher risk for developing cardiovascular disease presented by either coronary, cerebrovascular and/or peripheral arterial atherosclerosis, compared to age-matched controls [4,5]. It is a classical type 4 representative of the cardiorenal syndrome, a condition in which a single organ failure interrelates and affects the function of another organ [6,7].

The pathophysiology of this epidemical disease is both complex and multifactorial. Aside from the well-established traditional robust clinical predictors, such as age, arterial hypertension, hypercholesterolemia, obesity, diabetes mellitus, and hyperhomocysteinemia [4,8], novel mechanisms are being actively investigated. Endothelial dysfunction, vascular calcification, oxidative stress (OS), inflammation, and reduced nitric oxide (NO) availability all interplay in the progressive spiral of kidney function worsening [9,10].

Oxidative stress is the imbalance between reactive oxygen species (ROS) production and degradation. Clinical trials have been mostly investigating lipid peroxidation metabolites, such as 4-hydroxynonenal, 4-hydroxyhexenal, and malondialdehyde (MDA). Lipid peroxidation involves hydrogen abstraction from a carbon atom and oxygen insertion, resulting in the formation of peroxyl radicals and lipid hydroperoxides [11]. MDA, a colourless liquid with the nominal formula $CH_2(CHO)_2$, has often been utilised as a valuable biomarker of OS due to its relative chemical stability, which has been previously reported [12].

Although the effects of renal dysfunction on arterial disease are well documented and proven, the data on OS in the cohort of patients with severe atherosclerosis requiring open-heart surgical procedures are scarce. The artificial surface of the cardiopulmonary bypass machine, the

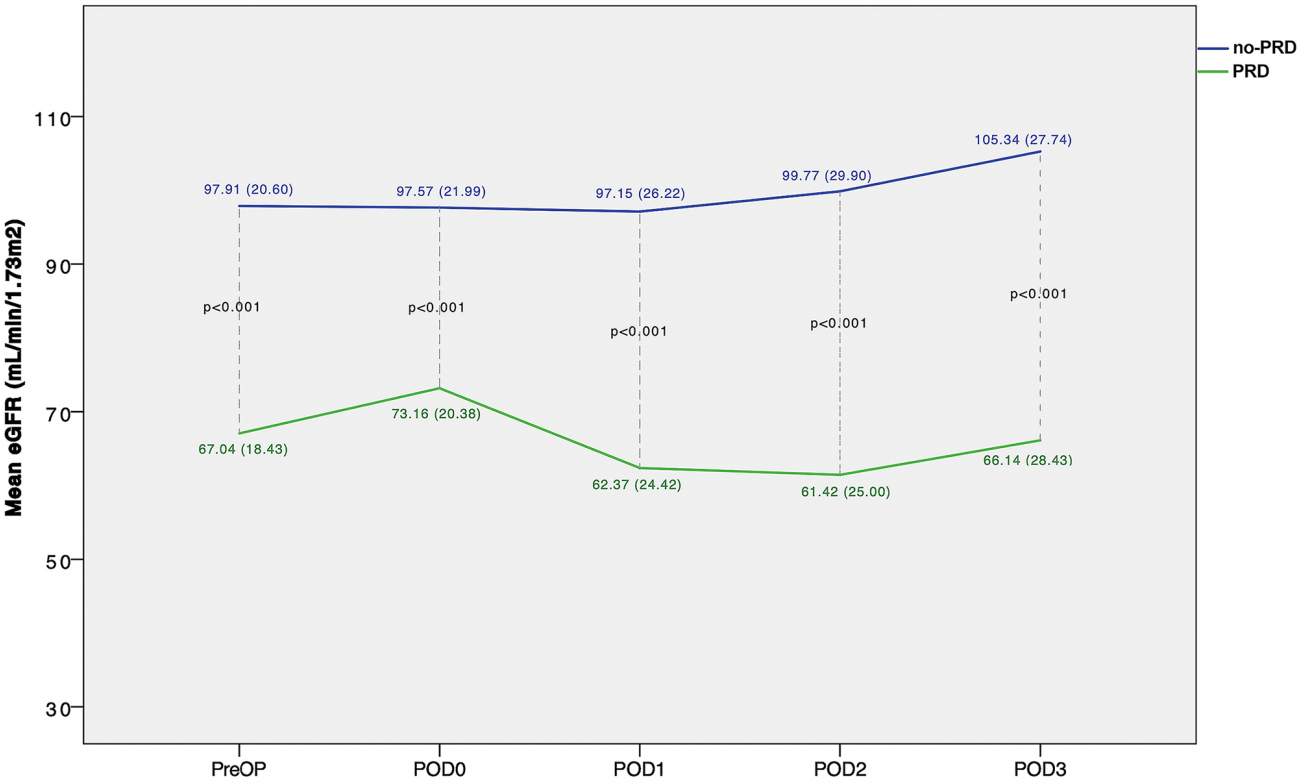

Fig. 1. Estimated glomerular filtration rate dynamics between preoperative renal dysfunction and normal function groups at various time points. PRD, preoperative renal dysfunction; no-PRD, normal preoperative renal function; eGFR, estimated glomerular filtration.

ischemic-reperfusion injuries and the side effects of certain medications, such as antibiotics or anti-hypertensive drugs, all play their role in ROS imbalance [13–15].

To the best of our knowledge, no study has been monitoring perioperative MDA concentrations in patients with preoperative renal dysfunction undergoing on-pump cardiac surgery using cardiopulmonary bypass to date.

The aim of this study was to compare the perioperative serum concentration of malondialdehyde as an indicator of lipid peroxidation in patients with preoperative renal dysfunction to patients with normal kidney function after on-pump cardiac surgery.

Materials and Methods

Study Sample

This prospective observational study was performed on elective patients who were scheduled for on-pump open-heart surgery at the Department of Cardiac Surgery at the University Medical Centre Maribor, Slovenia, between March 2019 and September 2019.

The exclusion criteria were patients under the age of 18, off-pump surgery, emergency, hypothermic circulatory arrest, preoperative chronic kidney disease on any kind of renal replacement therapy, and death before the completion of the study protocol (before the 3rd postoperative day (POD)).

Prior to enrolment, the patients were informed about the study protocol and a written informed consent was obtained. Based on the assessment of the preoperative kidney function through eGFR (calculated with the Modification of Diet in Kidney Diseases equation [16]), the patients were enrolled either to the preoperative renal dysfunction group (PRD) group or normal preoperative renal function (no-PRD) group.

Acute kidney injury was defined as accepted by Kidney Disease: Improving the Global Outcomes (KDIGO): an increase of serum creatinine of ≥26.5 μmol/L within 48 hours or more than a 50% baseline serum creatinine increase within 7 days [7].

Blood samples for the analysis of serum MDA levels were obtained preoperatively and intraoperatively immediately after the aortic clamp was removed. Postoperatively, blood samples were obtained 12 h after the surgery and then for the first three consecutive PODs.

Blood samples for the analysis of serum MDA levels were obtained at six different time points: prior to skin incision, intraoperatively immediately after the aortic clamp release, 12 h, 24 h, 48 h and 72 h after the procedure, respectively.

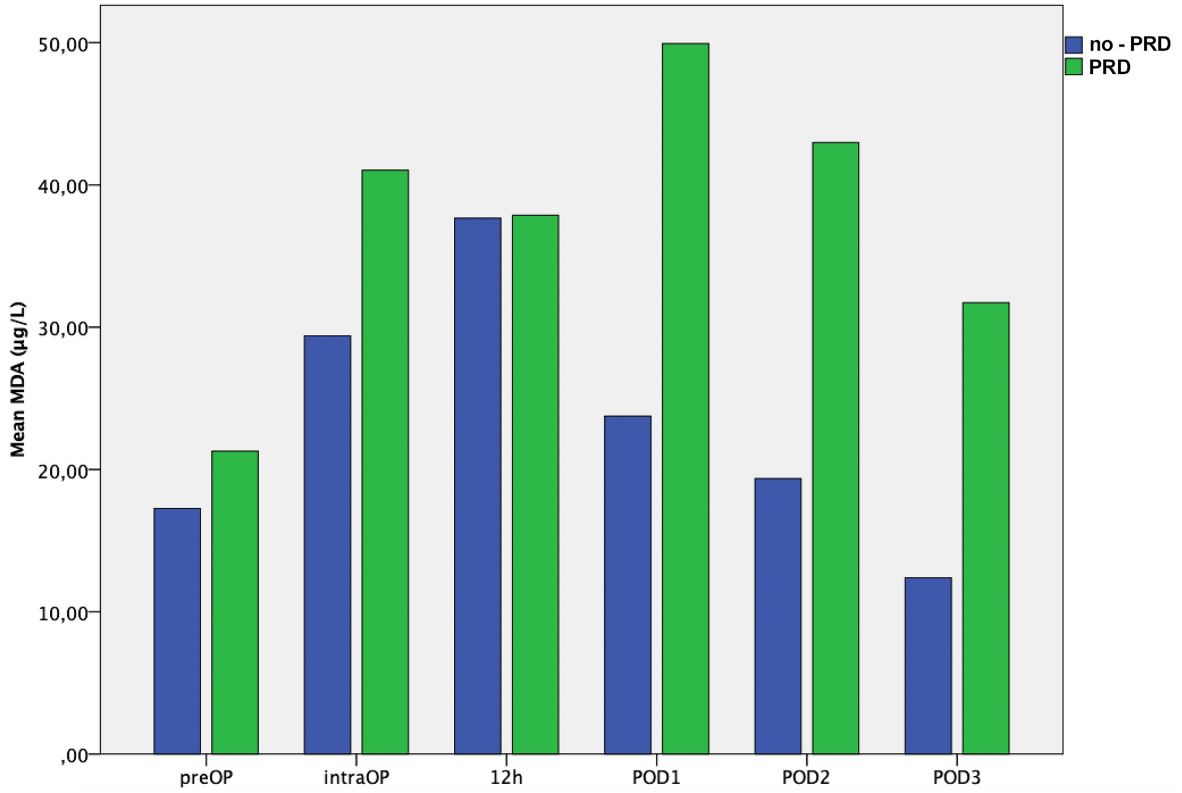

Fig. 2. Serum malondialdehyde concentrations dynamics between preoperative renal dysfunction and normal function groups at various time points. MDA, malondialdehyde.

Participant Characteristics

All enrolled participants were consecutive elective cardiac surgery patients who were operated on with the use of cardiopulmonary bypass and met none of the above-mentioned exclusion criteria.

Surgery

All patients underwent the same anaesthesia protocol and standard cardiac surgery with full sternotomy and cardiopulmonary bypass (CPB). Intraoperative data, such as CPB time, aortic cross-clamp time, urine output and fluid load, was documented. Postoperative creatinine levels were recorded on admission to the intensive care unit (ICU) and for the first 3 days after surgery. Both groups were subject to the same routine postoperative ICU and ward care.

Malondialdehyde Detection

After centrifugation, the derivatisation with pentafluorophenylhydrazine (PFPH) was carried out at 50 °C for 10 min in a closed vial. Helium was selected as the carrier gas, and the separation was achieved using a temperature gradient. The compounds were then detected using a mass detector [12].

Statistical Analysis

Normally distributed numerical data were expressed as the means (standard deviation, SD) and analysed using the Student's *t*-test. Non-normally distributed data were expressed as medians [interquartile range, IQR] and analysed using the Mann–Whitney U test. Categorical variables were summarised as frequencies (percentages) and compared using the chi-square test or Fisher exact test (when the expected value in the contingency table was <5).

After careful preselection of the variables based on univariate models and the results of our previous research [12], three linear regression models were constructed to assess the independent correlates to MDA levels at three time points (POD1, POD2 and POD3). Preoperative renal dysfunction, age, gender, aortic cross-clamp time, preoperative MDA level, and eGFR at the corresponding time point were chosen as independent variables in each regression model. All entered independent variables were tested for multicollinearity.

The sample size assessment was based on the presumption that the postoperative concentration of MDA in the PRD group would be around 30 percent points higher than in the no-PRD group. As there are no previous studies in this field, our last research data was used as an assumption [11].

Table 1. Preoperative patient characteristics.

	no-PRD (n = 28)	PRD (n = 87)	*p*-value
Age (years)	62 [12]	71 [15]	<0.001
Male sex	25 (89.3%)	58 (66.7%)	0.028
Body mass index (kg/m^2)	30.1 [11.2]	27 [6.8]	0.336
Diabetes	4 (14.3%)	22 (25.3%)	0.302
Arterial hypertension	17 (60.7%)	58 (66.7%)	0.388
History of myocardial infarction	4 (14.3%)	20 (21.8%)	0.341
History of stroke	1 (3.6%)	7 (8%)	0.677
Peripheral arterial obstructive disease	6 (21.4%)	25 (28.7%)	0.625
Redo surgery	0	2 (2.3%)	>0.999
EuroSCORE II (%)	1.02 [1.42]	2.28 [3.1]	<0.001
WBC count (10^9/L)	6.69 (1.45)	7.56 (2.22)	0.120
Haemoglobin concentration (g/L)	137.6 (16.0)	133.0 (16.5)	0.204
eGFR (mL/min/1.73 m^2)	97.91 (20.60)	67.04 (18.43)	<0.001
CKD staging:			
- no CKD	28 (100%)		
- stage 2		55 (63.2%)	
- stage 3		27 (31.0%)	
- stage 4		5 (5.7%)	
Beta-blocker	17 (60.7%)	62 (71.3%)	0.351
ACEI/ARB	16 (57.1%)	58 (66.7%)	0.373
Statin	18 (64.3%)	64 (73.6%)	0.348
Aspirin	20 (71.4%)	68 (78.2%)	0.454
Diuretic	2 (7.1%)	21 (24.1%)	0.059

EuroSCORE, European System for Cardiac Operative Risk Evaluation; eGFR, estimated glomerular filtration rate; WBC, white blood cell; PRD, preoperative renal dysfunction; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

With the enrolment ratio of 3:1, a power of 80%, beta error of 0.2 and alpha of 0.05, a total of 105 patients were required in the study (81 in the PRD group and 24 in the no-PRD group). Data analysis was performed using the IBM SPSS 25.0 software (IBM Inc., Armonk, NY, USA).

Results

A total of 116 patients were enrolled in the trial. One patient died before the completion of the study protocol and this patient was excluded from the study. Therefore, 115 patients were included in the final analysis. The PRD group consisted of 87 patients and the no-PRD group included 28 patients. Baseline demographics and comorbidities are presented in Table 1. There were more male patients in the no-PRD group (89.3% *vs*. 66.7%; $p = 0.028$). The patients in the PRD group were older compared to the no-PRD group (62 [12] years *vs*. 71 [15] years; $p < 0.001$). Expectedly, the EuroSCORE II was higher in the PRD group (2.28 [3.1] % *vs*. 1.02 [1.42] %; $p < 0.001$). Both groups had similar intraoperative characteristics with no significant differences in the type of surgery, CPB time, aortic cross-clamp time, intraoperative urine output, and fluid supplementation (Table 2). Patients in the PRD group had significantly lower eGFR compared to the patients in the no-PRD group ($p < 0.001$ at all time points) (Fig. 1). Perioperative kinetics of serum MDA concentration is presented in Fig. 2. There were no significant differences in the preoperative and intraoperative serum MDA levels ($p = 0.264$ and 0.148, respectively). Also, the study groups did not differ regarding serum MDA levels 12 hours postoperatively ($p = 0.981$). However, on POD1, POD2 and POD3 the serum MDA levels were significantly higher in the PRD group ($p < 0.001$ for POD1, POD2 and POD3). In the regression analyses, both preoperative PRD and preoperative plasma MDA concentration were shown to be an independent predictors of postoperative MDA levels at POD1, POD2 and POD3 ($p = 0.011$, 0.022 and 0.022, respectively). On the contrary, age, gender, aortic crossclamp time and eGFR at the corresponding timepoint could not be proven to be significant independent predictors of postoperative serum MDA concentration (Table 3).

From the 115 included patients, altogether 3 patients died during the hospitalization, all of them from the PRD group. The study groups did not differ in the ICU length of stay (no-PRD group 1.5 [2] days *vs*. PRD group 2 [2] days; $p = 0.296$). However, the patients in the PRD group had longer hospital stay (8 [2] days *vs*. 9 [3] days; $p = 0.037$). There were more patients in the PRD group who postoper-

Table 2. Intraoperative data.

	no-PRD (n = 28)	PRD (n = 87)	*p*-value
CPB time (min)	104.29 (24.22)	107.40 (37.53)	0.682
Aortic cross-clamp time (min)	84.21 (20.90)	83.68 (28.20)	0.927
Intraoperative urine output (mL)	364 (43)	307 (19)	0.174
Intraoperative fluid load (mL)	2341 (145)	2200 (87)	0.409
Valvular surgery			
Aortic	7 (25%)	27 (31.0%)	0.638
Mitral	7 (25%)	15 (17.2%)	0.410
Tricuspid	0	7 (8.0%)	0.192
CABG	17 (60.7%)	53 (60.9%)	>0.999
Aortic Surgery	4 (14.2%)	3 (3.4%)	0.059
Other	0	11 (12.6%)	0.063

CPB, cardiopulmonary bypass; CABG, coronary artery bypass grafting.

Table 3. Linear regression analyses.

Time point	Independent variable	B	Std. error B	*p* value	95% CI for B
POD 1	PRD	21.816	8.425	0.011	5.096∼38.535
	Age	0.297	0.361	0.413	–0.419∼1.014
	Sex	–3.812	7.045	0.590	–17.793∼10.169
	AXC time	–0.029	–0.120	0.810	–0.266∼0.209
	Preoperative MDA level	0.883	0.182	<0.001	0.522∼1.244
	eGFR (POD1)	0.121	0.128	0.347	–0.133∼0.376
POD 2	PRD	21.731	9.332	0.022	3.199∼40.263
	Age	0.380	0.409	0.355	–0.432∼1.191
	Sex	0.746	8.014	0.926	–15.168∼16.661
	AXC time	0.150	0.146	0.309	–0.141∼0.440
	Preoperative MDA level	0.482	0.211	0.025	0.063∼0.901
	eGFR (POD2)	0.075	0.135	0.579	–0.193∼0.344
POD 3	PRD	18.116	7.792	0.022	2.654∼33.578
	Age	–0.026	0.343	0.940	–0.706∼0.654
	Sex	–2.946	6.639	0.658	–16.120∼10.228
	AXC time	0.054	0.123	0.663	–0.190∼0.297
	Preoperative MDA level	0.415	0.177	0.021	0.065∼0.766
	eGFR (POD3)	0.032	0.105	0.764	–0.178∼0.241

POD, postoperative day; CI, confidence interval; PRD, preoperative renal dysfunction; AXC, aortic cross-clamp; MDA, malondialdehyde; eGFR, estimated glomerular filtration rate.

atively developed acute kidney injury (33.3% *vs*. 14.3%), however with a marginal significance ($p = 0.053$). There was only one patient who postoperatively required renal replacement therapy, the patient was from the PRD group.

We could not detect any significant correlation of a prolonged and intensified postoperative stress (indicated by the maximal postoperative MDA level) on selected clinical outcome markers such as postoperative eGFR, ICU stay, hospital stay or postoperative acute kidney injury incidence (Table 4, Ref. [7]).

Discussion

The results of this study indicate that cardiac surgery patients with preoperative renal impairment present with more extensive oxidative stress resulting in protracted lipid peroxidation in the early postoperative period compared to patients with a normal kidney function. Our previous study on 40 patients with a mean preoperative eGFR of more than 90 mL/min/1.73 m^2 [12] showed that the serum MDA concentration begins to increase during surgery and reaches peak values 12 hours postoperatively. Then, it starts to decline and reaches preoperative (baseline) values on the third postoperative day. Compared to the present study, these results are in accordance with the MDA kinetics of the no-PRD group but not the PRD group. In the latter, the MDA levels continued to rise and peaked about 12 hours later, i.e., on postoperative day 1, indicating a prolonged and intensified postoperative lipid peroxidation. Although the eGFR in the PRD group was significantly lower than the no-PRD group at all time points, the regression analyses have not

Table 4. Spearman's rank correlation between maximal postoperative MDA level and selected clinical outcomes.

	Correlation coefficient	*p*
ICU stay	–0.117	0.215
Hospital stay	0.008	0.931
Acute kidney injury*	–0.90	0.340
eGRF min**	–0.085	0.371

*as accepted by the Kidney Disease: Improving the Global Outcomes (KDIGO): an increase of serum creatinine of ≥26.5 µmol/L within 48 hours or more than a 50% baseline serum creatinine increase within 7 days [7]. **minimal postoperative eGFR. ICU, intensive care unit.

proved eGFR as an independent predictor of serum MDA concentration. This suggests a more complex relation between chronic kidney failure and oxidative stress that could be solely explained by the momentary creatinine excretion function of the kidneys. The MDA serum concentration did not significantly differ between the study groups until the first postoperative day, but afterwards, the patients in the PRD group presented with significantly higher values. We believe that this divergence in MDA kinetics indicates that patients with renal impairment might have a lower intrinsic antioxidant capacity compared to non-PRD patients. Although there is evidence that oxidative stress plays an important role in the etiology, pathophysiology, and progression of chronic kidney disease [4,9,17–19], the exact mechanism of how endogenous non-enzymatic and enzymatic antioxidants (such as superoxide dismutase, glutathione peroxidase or catalase) are affected by kidney dysfunction, remains poorly understood. Impaired function of the mitochondria (which are present vastly in the proximal tubules) in PRD patients is suggested both as the consequence and the cause of enhanced oxidative stress, which may explain a subnormal energy metabolism in this population [9].

Our study also has some limitations. Firstly, it is a small, single-centre, observational study which included only 115 patients. Secondly, the follow-up was only three postoperative days, limiting any information on how serum MDA levels affect mid- and long-term morbidity and mortality. Thirdly, the results of this study have not been confirmed using other methods for GFR estimation, e.g., the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20]. Fourth, although creatinine-derived markers have been generally accepted as good renal function indicators, more sensitive and earlier biochemical markers of renal dysfunction exist, including cysteine-rich protein 61, cystatin C, and Klotho [21,22].

Conclusions

To conclude, this is the first study investigating the amount of lipid peroxidation in PRD patients after open-heart surgery. Our results show that patients with PRD exhibit a more pronounced and prolonged oxidative stress, resulting in protracted lipid peroxidation in the early postoperative period compared to patients with normal kidney function. Further research focusing on the kinetics of oxidative stress, mechanisms of action, and potential benefits of antioxidant supplementation in this patient population is warranted.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

Conceptualization, MA and AD; methodology, MA and AD; formal analysis, MA; writing—original draft preparation, MA, AD and JK; writing—review and editing, MA, AD and JK; visualization, MA; supervision, MA and JK. All authors have read and agreed to the published version of the manuscript. All authors participated in data curation and revision of the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The study protocol was approved by the National Medical Ethics Committee of the Republic of Slovenia (Nr. 0120-268/2018/4) and is in full accordance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, *et al*. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney International. 2005; 67: 2089–2100.

[2] Antonic, M. Preoperative renal dysfunction is a predictor of postoperative atrial fibrillation in coronary artery bypass patients. Acta Medico-Biotechnica. 2019; 12: 29–38.

[3] Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. American Journal of Kidney Diseases: the Official Journal of the National Kidney Foundation. 1998; 32: S112–S119.

[4] Podkowińska A, Formanowicz D. Chronic Kidney Disease as Oxidative Stress- and Inflammatory-Mediated Cardiovascular Disease. Antioxidants (Basel, Switzerland). 2020; 9: 752.

[5] Srivastava A, Kaze AD, McMullan CJ, Isakova T, Waikar SS. Uric Acid and the Risks of Kidney Failure and Death in Individuals With CKD. American Journal of Kidney Diseases: the Official Journal of the National Kidney Foundation. 2018; 71: 362–370.

[6] Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. Journal of the American College of Cardiology. 2008; 52: 1527–1539.

[7] Djordjević A, Šušak S, Velicki L, Antonič M. ACUTE KIDNEY INJURY AFTER OPEN-HEART SURGERY PROCEDURES. Acta Clinica Croatica. 2021; 60: 120–126.

[8] Kao MPC, Ang DSC, Pall A, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. Journal of Human Hypertension. 2010; 24: 1–8.

[9] Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. Iranian Journal of Kidney Diseases. 2015; 9: 165–179.

[10] Luczak M, Formanowicz D, Pawliczak E, Wanic-Kossowska M, Wykretowicz A, Figlerowicz M. Chronic kidney disease-related atherosclerosis - proteomic studies of blood plasma. Proteome Science. 2011; 9: 25.

[11] Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, *et al*. Lipid Peroxidation-Derived Aldehydes, 4-Hydroxynonenal and Malondialdehyde in Aging-Related Disorders. Antioxidants (Basel, Switzerland). 2018; 7: 102.

[12] Djordjević A, Kotnik P, Horvat D, Knez Ž, Antonič M. Pharmacodynamics of malondialdehyde as indirect oxidative stress marker after arrested-heart cardiopulmonary bypass surgery. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2020; 132: 110877.

[13] Zhang WR, Garg AX, Coca SG, Devereaux PJ, Eikelboom J, Kavsak P, *et al*. Plasma IL-6 and IL-10 Concentrations Predict AKI and Long-Term Mortality in Adults after Cardiac Surgery. Journal of the American Society of Nephrology: JASN. 2015; 26: 3123–3132.

[14] Vermeulen Windsant IC, de Wit NCJ, Sertorio JTC, van Bijnen AA, Ganushchak YM, Heijmans JH, *et al*. Hemolysis during cardiac surgery is associated with increased intravascular nitric oxide consumption and perioperative kidney and intestinal tissue damage. Frontiers in Physiology. 2014; 5: 340.

[15] Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. Journal of the American Society of Nephrology: JASN. 2007; 18: 414–420.

[16] Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Annals of Internal Medicine. 1999; 130: 461–470.

[17] Daenen K, Andries A, Mekahli D, Van Schepdael A, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pediatric Nephrology (Berlin, Germany). 2019; 34: 975–991.

[18] Leyane TS, Jere SW, Houreld NN. Oxidative Stress in Ageing and Chronic Degenerative Pathologies: Molecular Mechanisms Involved in Counteracting Oxidative Stress and Chronic Inflammation. International Journal of Molecular Sciences. 2022; 23: 7273.

[19] Djordjevic A, Susak S, Kotnik P, Gorenjak M, Knez Z, Antonic M. Effect of Ascorbic Acid on Cardiac Surgery-Associated Acute Kidney Injury Incidence. The Thoracic and Cardiovascular Surgeon. 2022; 70: 566–574.

[20] Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, *et al*. A new equation to estimate glomerular filtration rate. Annals of Internal Medicine. 2009; 150: 604–612.

[21] Mosa OF, Skitek M, Kalisnik JM, Jerin A. Evaluation of serum cysteine-rich protein 61 and cystatin C levels for assessment of acute kidney injury after cardiac surgery. Renal Failure. 2016; 38: 699–705.

[22] Jerin A, Mosa OF, Kališnik JM, Žibert J, Skitek M. Serum Klotho as a marker for early diagnosis of acute kidney injury after cardiac surgery. Journal of Medical Biochemistry. 2020; 39: 133–139.