Effect of Ramadan Fasting on Malondialdehyde, Poly (ADP-Ribose) Polymerase, Sirtuin 1, Nuclear Receptor Subfamily 1 Group D Member 1, and Transforming Growth Factor Beta in Chronic Kidney Disease: A Prospective Cohort Study

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ABSTRACT

Background: *Chronic kidney disease (CKD) is a global health problem with increasing prevalence. This study aims to analyze the effect of Ramadan fasting on important biomarkers in CKD patients.* ***Methods:*** *A prospective cohort study was conducted on 30 CKD patients with stages 1, 2, and 3A who underwent Ramadan fasting. Measurements of MDA, PARP, SIRT1, NR1D1, and TGF-β levels were carried out before fasting, 2 weeks during fasting, and after fasting using the Enzyme-linked immunosorbent assay (ELISA) method.* ***Results:*** *There were significant decreases in urea, fasting blood glucose, HbA1C, and uric acid levels ($p<0.05$). MDA and SIRT1 decreased significantly ($p<0.001$), while PARP and NR1D1 increased significantly ($p<0.001$). TGF-β also showed a decrease. There were no significant changes in lipid profiles, creatinine, and albumin.* ***Conclusion:*** *Ramadan fasting has significant effects on several biochemical parameters and biological markers in early-stage CKD patients. These changes indicate potential improvements in oxidative stress, cell autophagy, inflammation regulation, and circadian rhythm. Further studies are needed to evaluate the long-term effects and clinical implications of these findings in CKD management.*

Keywords: *biomarkers, chronic kidney disease, inflammation, Ramadan fasting, oxidative stress.*

INTRODUCTION

Chronic kidney disease (CKD) is a global health issue with an increasing prevalence. According to the Indonesian Renal Registry, there has been a significant rise in the number of new CKD patients in Indonesia, from 25,446 in 2016 to 66,433 in 2018. In West Sumatra, the prevalence of CKD reached 0.2%, with the highest rates in Tanah Datar Regency and Solok City at 0.4%. CKD is characterized by a progressive decline in kidney function, eventually leading to kidney failure. Despite various therapeutic efforts, no definitive treatment for CKD has been found. Therefore, alternative approaches are needed to slow the progression of this disease.[1,2]

Ramadan fasting, as a form of calorie restriction, has shown various health benefits. Previous research indicates that Ramadan fasting can lower body mass index, fasting blood glucose, triglycerides, and inflammatory markers such as IL-1beta, IL-6, and TNF-α. Additionally, intermittent fasting has been proven to enhance antioxidant activity, DNA repair, and mitochondrial biogenesis.[3]

In CKD patients, there is an increase in oxidative stress and inflammation, which

contribute to kidney damage. Several biomarkers, such as Malondialdehyde (MDA), poly (ADP-ribose) polymerase (PARP), sirtuin 1 (SIRT1), nuclear receptor subfamily 1 group D member 1 (NR1D1), and transforming growth factor beta (TGF-β), have been identified as important markers in CKD pathogenesis. MDA is an indicator of oxidative damage that increases with the progression of kidney dysfunction. PARP plays a role in DNA damage and inflammatory responses in kidney tissue. SIRT1 has antifibrotic functions and plays a role in protecting kidney tissue. NR1D1 is involved in the response to oxidative stress and DNA damage, while TGF-β is a key pro-fibrotic factor in kidney fibrosis.[4]

Although the benefits of Ramadan fasting have been widely studied in healthy individuals, its impact on CKD patients remains debated. Therefore, this study analyzed the effect of Ramadan fasting on the levels of MDA, PARP, SIRT1, NR1D1, and TGF-β in CKD patients. The results of this study are expected to provide new insights into the potential of Ramadan fasting as a therapeutic approach in CKD management and to assist in clinical decision-making regarding the practice of Ramadan fasting by CKD patients.

METHODS

This is a prospective cohort study conducted from January 2024 to August 2024 at the Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang, the special polyclinic at Dr. M. Djamil General Hospital, and the internal medicine clinic at a private hospital in Padang. The study population consisted of CKD patients attending the special clinic at Dr. M. Djamil General Hospital and the internal medicine clinic at the private hospital in Padang. A sample of 30 CKD patients was selected, meeting the inclusion and exclusion criteria.

The inclusion criteria were as follows: adult (aged >18 years), CKD at stage 1, 2, or 3A, regularly fasted during Ramadan for at least the past year, and willing to participate in the study. The exclusion criteria included: patients with type 1 diabetes mellitus, patients with severe comorbidities in the past 3 months, and patients who have undergone hemodialysis.

Statistical Analysis

The independent variable in this study is the group of CKD patients who fasted for at least 15 days during Ramadan. The dependent variables are the expression levels of PARP, SIRT1, NR1D1, and TGF-β. Levels of MDA, PARP, SIRT1, NR1D1, and TGF-β were measured using the ELISA method. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula based on creatinine tests. The normality of the data was tested using the Shapiro-Wilk test. If the data were normally distributed, a parametric repeated ANOVA test was used for analysis. If the data were not normally distributed, data transformation was performed. If the data remained non-normally distributed, the Friedman test was used. Significance was determined at $p<0.05$.

Ethical Clearance

This study was approved by the Ethical Committee of M. Djamil Hospital, Padang (reference number: DP.04,03/D.XVI. XI/127/2024).

RESULTS

A total of 30 participants were included in this study, consisting of 19 males (63.3%) and 11 females (36.7%), with a mean age of 48.9 years. **Table 1** presents a comparison of laboratory parameters before and after fasting.

There were no significant changes in hemoglobin, hematocrit, leukocyte count, or platelet count ($p > 0.05$). Urea levels decreased significantly after fasting ($p = 0.03$), while creatinine levels did not show a significant difference ($p = 0.196$). No significant changes were observed in lipid profile components, including LDL ($p = 0.95$), HDL ($p = 0.76$), triglycerides ($p = 0.81$), and total cholesterol ($p = 0.69$).

Fasting blood glucose ($p < 0.01$) and HbA1c ($p < 0.01$) levels showed statistically significant reductions following the fasting period. Albumin levels remained unchanged ($p = 0.46$). In contrast, uric acid levels significantly decreased after fasting ($p = 0.02$).

Table 1. Comparison of Laboratory Parameters Before and After Fasting

Variable	Normality test (p)		Analysis test	
	Before fasting	After fasting	Paired t-test (p)	Wilcoxon (p)
Hb	0.793	0.178	0.55	
Ht	0.513	0.138	0.46	
Leuco	0.042	0.070		1
Thrombo	0.178	0.027		0.25
Ur	0.021	0.002		0.03
Cr	0.325	0.035		0.196
LDL	0.423	0.237	0.95	
HDL	0.473	0.578	0.76	
TG	0.008	0.014		0.81
Total Chol	0.040	0.016		0.69
GDP	<0.001	0.011		<0.01
HbA1C	0.003	0.007		<0.01
Alb	<0.001	0.004		0.46
UA	0.727	0.759	0.02	

In addition to routine laboratory parameters, molecular markers were also measured at three time points: before fasting, after 2 weeks of fasting, and after 1 month of fasting. As shown in **Figure 1-5**, a significant reduction was observed in malondialdehyde (MDA) levels over time ($p < 0.001$), indicating a decrease in oxidative stress. Conversely, poly (ADP-ribose) polymerase (PARP) levels significantly increased following the fasting period ($p < 0.001$). Sirtuin 1 (SIRT1) levels showed a significant decrease ($p < 0.001$), while nuclear receptor subfamily 1 group D member 1 (NR1D1) levels significantly increased ($p < 0.001$). Additionally, transforming growth factor beta (TGFβ) levels were significantly reduced after 1 month of fasting ($p < 0.001$).

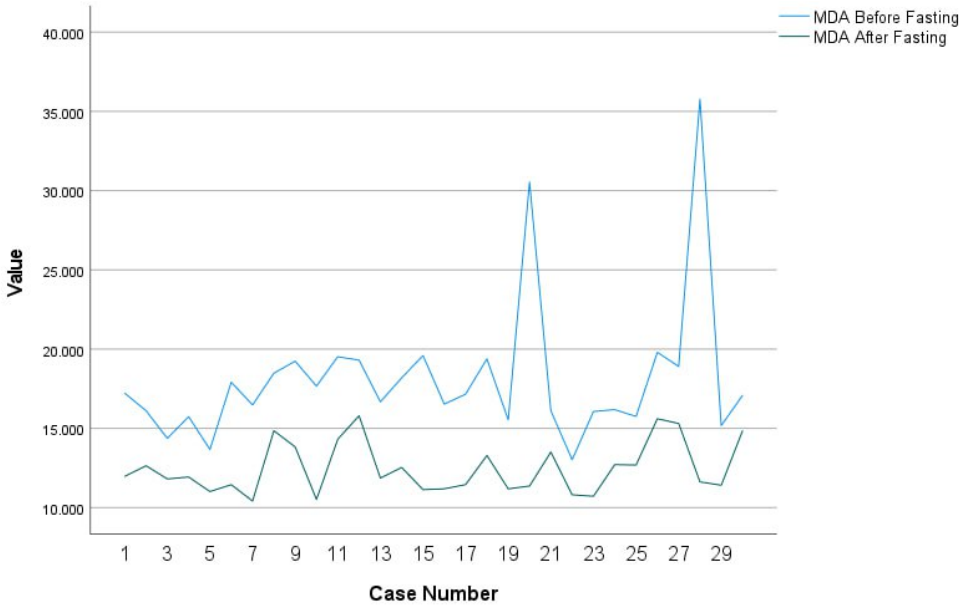

Figure 1. Graph of Differences in MDA Levels

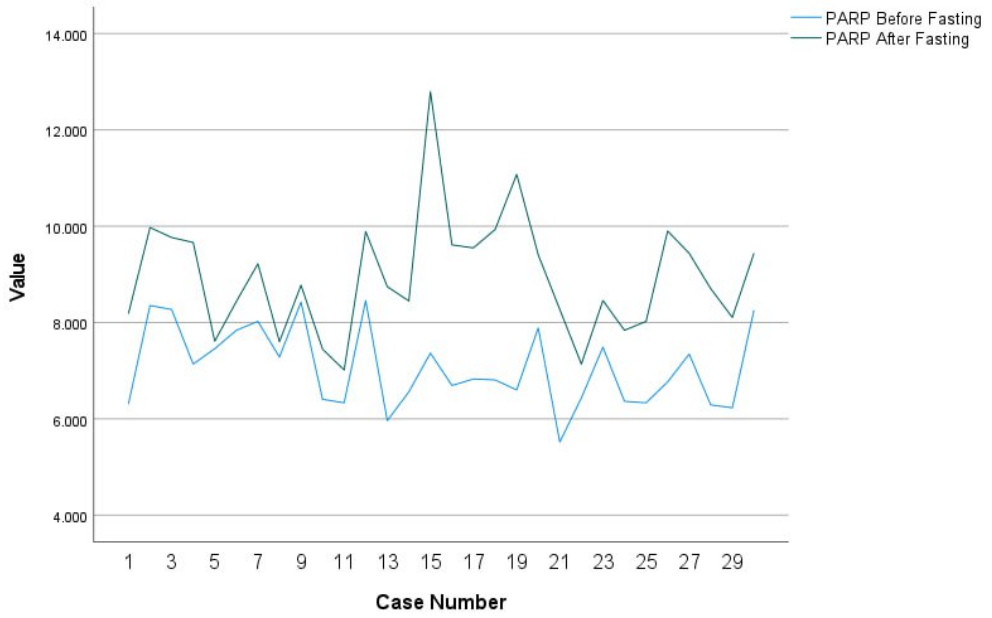

Figure 2. Graph of Differences in PARP Levels

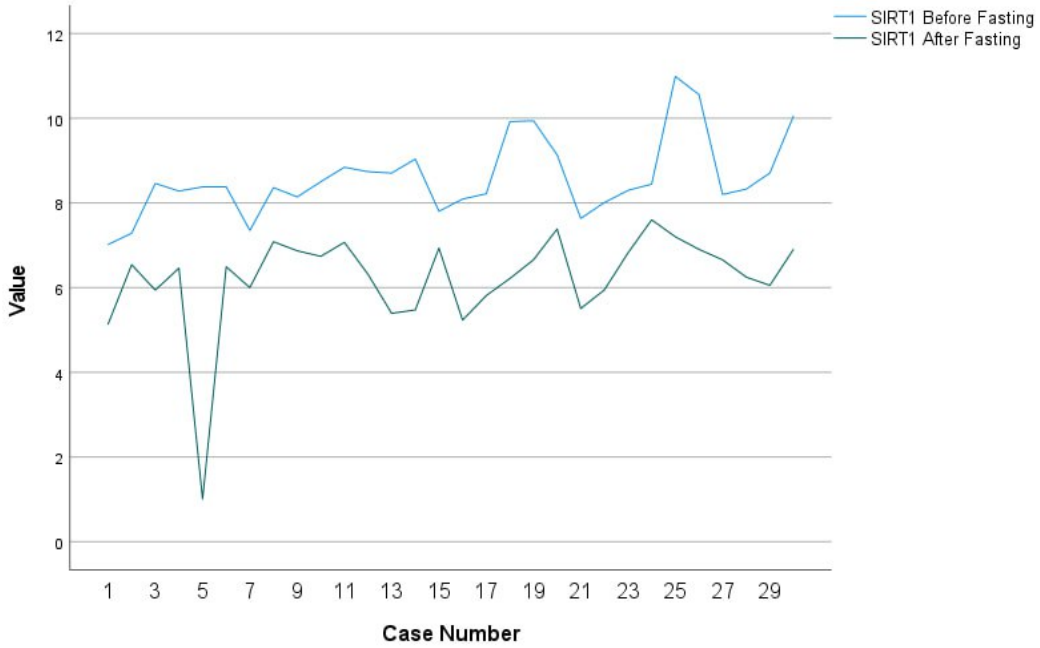

Figure 3. Graph of Differences in SIRT1 Levels

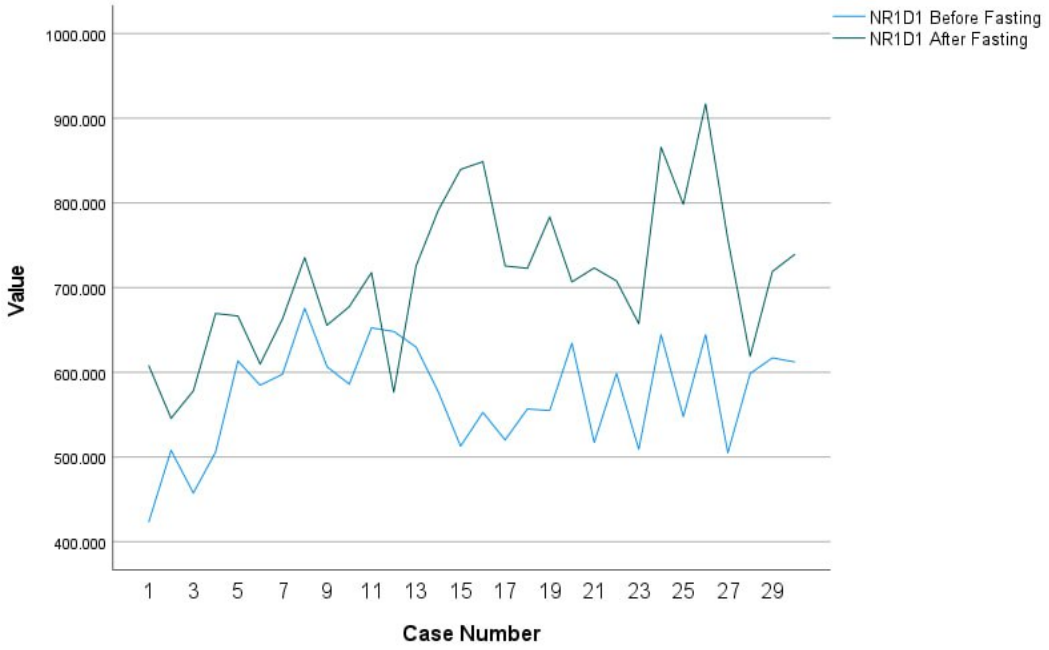

Figure 4. Graph of Differences in NR1D1 Levels

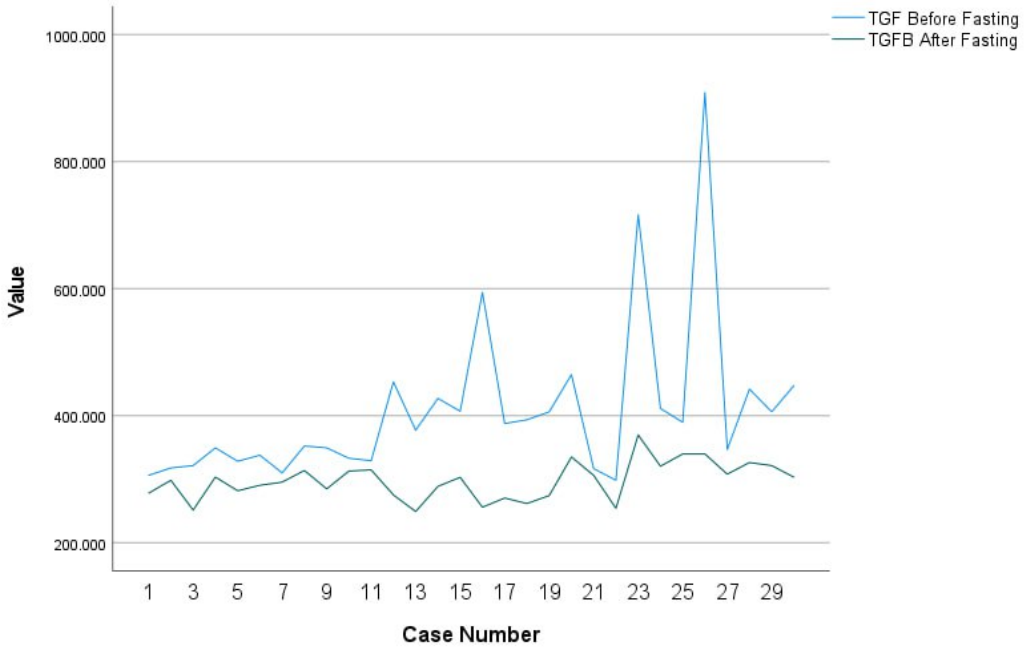

Figure 5. Graph of Differences in TGF-β Levels

DISCUSSION

In this study, the majority of the 30 CKD patients were male, with 19 men (63.3%) and 11 women (36.7%), which contrasts with global findings, where female patients tend to be more prevalent. According to Kovesdy ,[1] the global prevalence of CKD has been rising yearly. Based on 33 representative studies, it was found that 10.4% of men and 11.8% of women had CKD. In high-income countries, the prevalence is 8.6% for men and 9.6% for women, while in low-income countries, the prevalence is 10.6% for men and 12.5% for women.

The average age of CKD patients in this

study was 48.9 (±6.03) years. Global data on the average age of CKD patients are limited. Hill et al. analyzed the prevalence of CKD stages 1-5 across various age groups, finding that 13.7% were between 30 and 40 years old, while 27.9% were over 70 years old.[2]

Routine blood tests performed on the study subjects showed results within the normal range both before and after Ramadan fasting. Urea levels decreased from a median of 40 mg/dL (range: 21-87 mg/dL) to 2.5 mg/dL (range: 20-77 mg/dL). However, creatinine levels remained unchanged, with a median of 1.4 mg/dL. The impact of Ramadan fasting on urea and creatinine levels remains debated. In a study from Morocco by Mbarki et al. conducted during Ramadan 2010, 60 CKD patients fasted for nearly the entire month.[3] Of these, 11.7% experienced acute kidney failure, defined as a serum creatinine increase of ≥5 mg/L from baseline, a ≥50% rise in serum creatinine, or a ≥25% decrease in glomerular filtration rate (GFR) from baseline.

Nasrallah conducted a larger study involving 106 CKD patients and reported several serious side effects of fasting, including a 60.4% increase in serum creatinine by the seventh day and six cardiovascular events in the fasting group (two cases of acute coronary syndrome, two exacerbations of chronic peripheral vascular disease requiring surgery, one case of severe heart failure, and one minor non-haemorrhagic stroke).[4] Most of these patients (five out of six) had pre-existing cardiovascular conditions.

Conversely, other studies have shown different outcomes. For example, Hakim et al. (201) conducted a prospective observational cohort study of 31 CKD patients (14 with stage 3 CKD, 12 with stage 4 CKD, and 5 with stage 5 CKD).[5] The study showed a significant increase in eGFR from 29.7 mL /min/1.73 m² before fasting to 32.7 mL/min/1.73 m² after fasting, along with improved blood pressure control, better lipid profiles, and decreased urinary protein excretion.

Chowdhury studied fasting during the summer in London for a group of Muslims with stage 3 CKD during Ramadan 2018, where the fasting duration was 19 hours. The study compared 68 fasting patients with 71 non-fasting patients and found no significant changes in weight, blood pressure, creatinine, HbA1c, cholesterol, or urine protein-creatinine ratio before and after Ramadan. Additionally, there were no significant differences in worsening renal function, hypoglycemia, or cardiovascular events between the fasting and non-fasting groups.[6]

Mohajeri et al. studied the impact of Ramadan fasting on lipid profile parameters in healthy individuals aged 20-40 years.[7] They found no significant changes in hematological parameters after fasting. Similarly, Tashkandi et al.[8] showed that plasma lipid profiles changed during the study, with significantly higher total cholesterol (184 ± 48 vs. 169 ± 55 mg/dL), HDL (40 ± 14 vs. 36 ± 10 mg/dL), and LDL (114 ± 39 vs. 104 ± 45 mg/dL) two months after Ramadan, although triglyceride levels were not significantly affected (162 ± 55 vs. 143 ± 104 mg/dL).[8] This differs from the results of this study, where lipid profiles showed significant reductions in LDL, triglycerides, and total cholesterol. However, in this study, lipid profile measurements in CKD patients who fasted during Ramadan showed a decrease in mean LDL, triglycerides, and total cholesterol levels, along with an increase in HDL, though these changes were not statistically significant.

Diabetes in CKD patients increases the complexity of management. To ensure appropriate decision-making and improve patient safety, the Diabetes and Ramadan risk calculator, which considers clinical parameters such as kidney function, has been utilized to assess fasting risks and offer personalized recommendations. Bakhit et al. studied 65 CKD patients who fasted during Ramadan and found improvements in glucose metabolism, with the average HbA1C levels decreasing from 8.2% to 7.3% after Ramadan fasting.[9] Similarly, in this study, the median HbA1C decreased from 7.15% (range: 5.4%-6.1%) before fasting to 7.10% (range: 5.2%-13%) after Ramadan fasting. Fasting blood glucose also showed a decrease in median values from 130 mg/dL (range: 87-380 mg/dL) before fasting to 122 mg/dL (range: 138-280 mg/dL) after fasting.

Increased uric acid in CKD patients is

a physiological process, and high uric acid levels can accelerate kidney function decline, potentially leading to kidney failure. Erdem et al. reported elevated uric acid levels in CKD patients above normal values.[10] Karatas et al. found significant differences in blood glucose, HbA1C, and uric acid levels in CKD patients before and after Ramadan fasting.[11]

Raju et al.[12] studied 28 adult CKD patients aged 18-70 years who fasted during Ramadan and analyzed various metabolic parameters. They found no significant difference in serum albumin levels before and after fasting, indicating that CKD patients could tolerate the metabolic demands of fasting, as found in this study.[12]

The median MDA levels in CKD patients before fasting were 17.12 nmol/mL (range: 13.02-35.77 nmol/mL). After two weeks of fasting, MDA levels decreased to a median of 15.03 nmol/mL (range: 12.4-18.35 nmol/mL), and by the end of Ramadan fasting, the median MDA level dropped further to 11.90 nmol/mL (range: 10.4-15.79 nmol/mL). Ibrahim et al. studied the effects of Ramadan fasting on oxidative stress and cellular damage in adults, finding that MDA levels decreased from 2.62 (0.23) to 2.4 (0.16) after 28 days of fasting.[13] However, MDA levels in this study were higher than those found in Ibrahim's study. Vodosek et al. mentioned that oxidative stress could be caused by various factors, including lipid peroxidation from free radicals.[14] MDA, formed during lipid peroxidation and the synthesis of prostaglandins and thromboxanes, can attack macromolecules and impair their function. Increased MDA levels are found in CKD patients compared to normal subjects, with a negative correlation to GFR and a significant correlation with CKD severity.

PARP plays a critical role in repairing damaged DNA and gene expression. In CKD patients, PARP activation is elevated compared to normal levels (<1.22 ng/mL), likely due to kidney cell damage that stimulates cellular autophagy to maintain cell cycle balance. In this study, PARP levels increased after Ramadan fasting, from a baseline mean of 7.06 (0.82) ng/mL to 7.93 (0.98) ng/mL after two weeks of fasting, and further to 8.94 (1.22) ng/mL by the end of Ramadan. The increase in PARP after fasting is attributed to starvation-induced autophagy, a process activated during fasting, optimizing cellular repair. Jose et al. emphasized that autophagy is the primary mechanism for reallocating nutrients from non-essential processes to critical functions during periods of starvation, as reflected by increased PARP activity in this study.[15]

SIRT1 acts as a marker for cellular regulation processes. Before fasting, CKD patients had a median SIRT1 level of 8.38 ng/mL (range: 7.02-10.99 ng/mL), which decreased to a median of 7.29 ng/mL (range: 5.36-9.89 ng/mL) after two weeks of fasting, and further to 6.47 ng/mL (range: 1.00-7.60 ng/mL) after Ramadan. Kilic et al. reported that SIRT1 levels increase with aging and inflammation, with normal SIRT1 levels at 1.58 (0.07) ng/mL in children, 1.84 (0.10) in adults, and 4.07 (0.22) in the elderly.[16] The elevated SIRT1 levels in CKD patients found in this study compared to normal individuals are likely due to inflammation and oxidative stress. Fasting led to a reduction in SIRT1 levels, suggesting that fasting may help prevent aging and regulate inflammation in CKD patients. This differs from Safitri et al. , who found that intermittent fasting (16:8) in diabetic rats reduced SIRT1 levels compared to non-fasting diabetic rats, possibly due to hyperglycemia suppressing SIRT1 activity.[17]

NR1D1 is an important regulator of circadian rhythms. In this study, the mean NR1D1 level in CKD patients before fasting was 573.14 (62.35) ng/L, increasing to 637.15 (66.51) ng/L after two weeks of fasting and further to 711.75 (89.28) ng/L by the end of Ramadan. NR1D1 values vary by individual and are higher in adults than in children. Research on NR1D1 in CKD patients is limited, and reference values are not well-established. Egstrand et al. found that NR1D1 expression significantly decreased in uremic rats with partial nephrectomy, potentially due to NR1D1's modulation of inflammatory macrophage functions through CCL2 gene expression.[18] Kashyap et al. mentioned that CCL2 is mechanistically linked to the pathogenesis of many inflammatory diseases, cardiovascular diseases, atherosclerosis, and CKD.[19] Pharmacological inhibition of CCL2

reduces kidney damage, suggesting that the dysregulation of NR1D1 may be linked to CKD progression.

The median TGF-β level in CKD patients before fasting was 382.45 pg/mL (range: 298.35-908.95 pg/mL), decreasing to 325.65 pg/mL (range: 271.37-763.82 pg/mL) after two weeks of fasting, and further to 300.69 pg/mL (range: 249.21-369.67 pg/mL) by the end of Ramadan. TGF-β levels in CKD patients were higher compared to the normal average of 108.14 (39.97) pg/mL, likely due to the inflammation associated with CKD. Ferucci et al. found that fasting for 12 hours led to a significant decrease in TGF-β (p=0.014), and Mehrabani et al. suggested that fasting activates mitochondrial splitting, enhancing the cell's ability to perform mitophagy, thereby improving cellular homeostasis.[20,21]

However, this study has limitations, such as not assessing the types of food or beverages consumed by patients, evaluating their activity levels before and during fasting, or conducting long-term follow-up after Ramadan. The novelty of this study is the finding that Ramadan fasting can improve inflammatory responses in CKD patients in stages 1-3a, as indicated by significant changes in MDA, SIRT1, TGF-β, PARP, and NR1D1 levels.

CONCLUSION

Ramadan fasting has been shown to reduce MDA, SIRT1, and TGF-β levels while increasing PARP and NR1D1 levels in patients with CKD stages 1 to 3a. However, Ramadan fasting does not significantly affect kidney function in patients with CKD stages 1 to 3a. Further research at a larger scale is needed to explore the effects of Ramadan fasting on patients with CKD across all stages, as well as involving nutritional assessment, patient activity evaluation, and longer evaluation periods.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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