Clinical and Experimental Health Sciences

Determination of Early Diagnostic Biomarkers of Renal Dysfunction After Cardiopulmonary Bypass: miR-21 and miR-10a Mediated Postoperative Inflammation

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ABSTRACT

Objective: Acute renal failure (ARF) prevalence is high among patients who undergo cardiopulmonary bypass (CPB), and this condition can only be diagnosed via serum creatinine level (sCr) conventionally within 48 hours. Therefore, we need early novel diagnosis biomarkers to start preventive treatment of ARF. For that reason, we aimed to analyze if plasma miR-21 derived from heart, correlates with kidney-enriched miR-10a during inflammatory IL-6, IL-1β, and TNF-α response in terms of acute renal failure 30 minutes after CPB.

Methods: Patients (n=46, Female:8 and Male:38), aged 61.08±9.41, who underwent CPB surgery were included. Blood samples were collected during the pre – and post-CPB (30 minutes after CPB). Demographic data of all cases were collected. Quantification of expression levels of miR-21 and miR-10a was done via quantitative PCR (qPCR). Determination of plasma concentration of relevant cytokines, IL-6, IL-1β, and TNF-α was done via ELISA.

Results: The circulating level of miR-21 during post-CPB period (-11.78±6.98) was significantly higher ($p \leq 0.05$) than pre-CPB period (-6.55±7.11), but there was no significant change ($p > 0.05$) in the circulating level of miR-10a between pre – (-12.22±3.55) and post-CPB (-11.60±3.36) periods. When we compared the mean ΔΔCt values of miR-21 and miR-10a, downregulation was observed in the expression level of miR-10a (0.62±3.77) whilst the expression level of miR-21 (-5.22±7.25) was upregulated ($p \leq 0.05$). The levels of plasma concentration of IL-6 (2.74±2.50 ng/l) and TNF-α (83.63±9.33 ng/l) were increased during post-CPB period (both were ***$p < 0.0001$). Whilst, IL-1β concentration level during pre-CPB period (3.95±0.47 ng/l) was found to be decreased (0.38±2.04 ng/l and *$p < 0.05$) according to post-CPB.

Conclusion: Prospectively, these data suggests that high miR-21 levels is a promising indicator and can be a candidate as an early novel biomarker for diagnosis of acute renal failure 30 minutes after CPB.

Keywords: MiR-21, miR-10a, biomarker, cardiopulmonary bypass, cytokines, inflammation, renal dysfunction

1. INTRODUCTION

Acute renal failure (ARF) is manifested by acute tubular necrosis of the renal tissue in 45% of hospitalized patients after operations done with CPB (1). If individuals with pre-existing renal failure are exposed to CPB, these patients need dialysis as a result of chronic renal failure (2). Renal failure is a complex syndrome and can be diagnosed via conventionally used method which is the increase in serum creatinine level (3). Acute renal failure that may develop after CPB, can be diagnosed 48 hours after the onset of damage (4). Therefore, serum creatinine level is not sufficient as a clinical early diagnosis biomarker (5). On the other hand, it has been suggested that acute inflammation is one of the triggering factors of damage that causes acute renal failure (6). During CPB, inflammation is triggered due to the reactions that occur as a result of the contact of blood with unknown surfaces (7). In these reactions, inflammatory mediators like cytokines are involved and the promotion of IL-6, IL-1β, and TNF-α (8). How inflammation after CPB links to organ failure remains unknown.

Under normal conditions, during the controlled up-regulation of miR-21 in cardiac tissue, it acts as a protective factor. Excessive up-regulation of miR-21 leads to tissue damage. These two opposing effects of miR-21 lead to its definition as a “double-edged sword” in the literature. It has been shown that cardiac MiR-21 is up-regulated through IL-6 release

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depending on the severity of inflammation and exacerbates tissue fibrosis, leading to chronic renal damage (9, 10).

MiR-10a is down-regulated due to inflammation. Thus, it has been known as a negative regulator of proinflammatory cytokines. Moreover, the downregulation of miR-10a is mediated via IL-1β, and TNF-α cytokines. MiR-10a-mediated activation of NF-kB stimulates the production of many cytokines (11, 12).

To the best of our knowledge there has been no association shown between several cytokines, miR-21 and miR-10a renal failure shortly after CPB. Possible inflammatory pathway regulated by IL-6, IL-1β, and TNF-α may cause renal failure after CPB is shown in Figure 1.

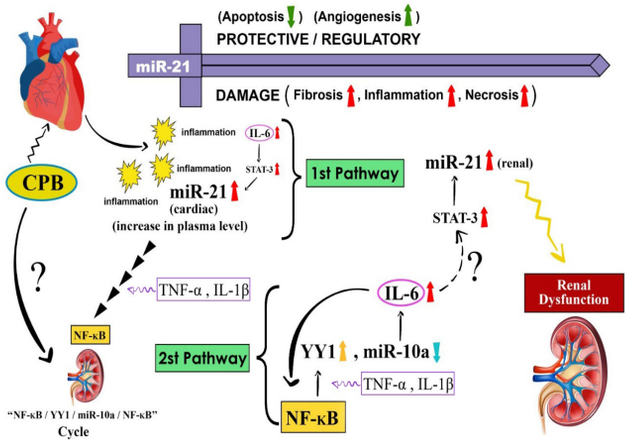

Figure 1. *Possible renal dysfunction biomarkers in patients after CPB; possible biochemical mechanism of miR-21 and miR-10a mediated postoperative inflammation. This figure is drawn by Çağıl F.Z.*

We illustrated that in the heart tissue during post-CPB period, miR-21 is excessively upregulated and released into the blood circulation and reaches kidneys where blood is being filtered. And it initiates the miR-10a / NF-kB cycle and cytokine release in renal tissue. We hypothesized that the trigger/initiator of this damaging mechanism could be miR-21 (1st pathway in Figure 1). In addition, the downregulation of miR-10a in renal tubules is mediated via IL-1β and TNF-α and induces IL-6 release. The increase in the level of IL-6 may also increase the level of renal miR-21. Together with the cardiac miR-21, this cycle becomes a continuous damaging mechanism (2nd pathway in Figure 1). For that reason, we studied plasma miR-21, miR-10a, IL-6, IL-1β, and TNF-α expressions for acute renal failure 30 minutes post CPB.

2. METHODS

2.1. Study Population and Design

Research Ethics Committee Approval is obtained from Marmara University (Protocol Number: 09.2019.859). All participants have been informed consents. All patients (n=46, Female:8 and Male:38), aged between 52 and 70 61.08±9.41, who underwent elective CPB surgery at Marmara University, Pendik Research and Training Hospital, School of Medicine, Cardiovascular Surgery Department between the dates 08/16/2021 and 11/15/2021 were included in the study. Patients who had abnormal pre-operative serum cardiac troponin (cTnI) values, patients applied heparin pre-operatively, and cases with combined heart surgery were not included in the research.

2.2. Collection of Blood Samples for Cytokine Analysis

To determine concentration levels of cytokines; 5 mL of blood samples were collected into vacuum tubes containing 3.2 % sodium citrate (BD Vacutainer) during pre – and post-CPB period. The blood samples taken were centrifuged at 2500 *rpm* for 20 minutes at 4 °C. Plasma samples were put in tubes as 500 µL aliquots. Then, aliquots were stored at – 80 °C for further use of downstream reactions.

2.3. Determination of Cytokine Level via ELISA

IL-6, IL-1β, TNF-α levels were measured via commercially available sandwich ELISA Kits (Catalog numbers are EK710267, EK710260, EK710127, respectively) (AFG Scientific, Northbrook, IL). Each sample was run in duplicate. Wash buffer was prepared. Wash buffer was warmed to get rid of crystals in the concentrate. Standard solution was diluted. Citrate treated plasma was thawed. 50 µL of standard was put in each well. Then, sample diluent was added in each well. After that 10 µL of sample was pipetted in wells in duplicates. All wells are covered and incubated for 30 min at 37°C. All wells are washed with wash buffer 5 times. Then, HRP-Conjugate reagent added in each well, except blank well. All wells are covered and incubated for 30 min at 37°C. All wells are washed with wash buffer 5 times. Color reaction was done with 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B to each well. At this step wells are avoided light and kept for 15 min at 37°C. Stop reaction was done via 50 µL of stop solution. Color change was obtained from blue to yellow. Plasma concentration of cytokines was measured at a wavelength of 450 nm absorbance via ELISA Reader, (Rayto Rt-2100c).

2.4. Collection of Blood Samples for Quantification of miR-21 and miR-10a

5 mL of blood samples were collected into vacuum tubes containing K2 EDTA 7.2 mg (BD Vacutainer). The blood samples were centrifuged at 2500 *rpm* for 20 minutes at 4 °C. Plasma samples were put in aliquots as 500 µL. Then, stored at – 80 °C for further use of downstream reactions.

2.5. Quantification of miR-21 and miR-10a via qRT-PCR

2.5.1. RNA Isolation

Total RNA was extracted from 500 µl EDTA treated plasma samples using 1000 µL Trizol-TRI Reagent according to the manufacturer's instructions (Merck/Sigma Aldrich). The RNA

was stored at – 80 °C for later analysis. The purity of RNA was assessed according to 260/280 ratio of absorbance value using the NanoDrop 2000 Spectrophotometer. The RNA concentration was calculated by multiplying the absorbance value and multiplying the RNA coefficient and the dilution coefficient:

RNA concentration (µg/mL) = OD at 260 nm x CC x 40 µg/mL

where: OD = optical density, nm = nanometer, CC = coefficiency coefficient, and an absorbance of 1 Unit at 260 nm corresponds to 40 µg of RNA per mL (A260 = 1 = 40 µg/mL).

2.5.2. Reverse transcription and quantification of miR-21 and miR-10a via qRT-PCR

Complementary DNA (cDNA) synthesis from total RNA was performed using the miRNA All-In-One cDNA Synthesis Kit (AbmGood, Catalog No: G898) according to the manufacturer's protocol. Sequences of primers are listed in Table 1. Expression of miR-21 and miR-10a were obtained as cycle threshold (Ct) values. All samples were run in duplicates. Mean values of Ct values were used for the calculation. The expression difference between pre-operative and post-operative mean ΔΔCT values was calculated as Mean of [ΔΔCT=ΔCT (a miRNA of post-CPB (30 minutes after CPB))-ΔCT (miRNA of pre-CPB as a normalizer accounting for sample to sample variation)]. To analyze the relative expression of miR-21 and miR-10a the fold change (relative quantification) was calculated via the $2^{-\Delta\Delta CT}$ formula (13).

Table 1. *Primer sequences designed specifically for miR-21 and miR-10a*

Primer	5'-3' primer sequence
hsa-miR-21-5p (Forward)	*GCAACCGGTAGCTTATCAGACTGATGT*
hsa-miR-10a-5p (Forward)	*GCAACCACTTACCCTGTAGATCCGAAT*
Universal Revers primer	*CAGTGCAGGGTCCGAGGTCAGAGCCACCT*

2.6. Statistical Analysis

The GraphPad Prism 5.0 software (GraphPad, San Diego, CA) was used for statistical analysis. The student's t-test was used to compare the mean values of groups. Non-parametric comparisons were done using the Mann-Whitney u test. Descriptive results of continuous variables are expressed as mean ±SE. Pearson correlation analysis was used to analyze the relation of miR-21 and miR-10a expression levels with IL-6, TNF-α, and IL-1β. Statistical differences between the pre-operative period and the postoperative period were analyzed. p ≤0.05 level was considered as statistically significant.

3. RESULTS

Clinical and demographic parameters are given in Table 2. The number of male cases (n=38) was higher than females (n=8). There was no significant difference between the ages of males and females (p>0.05). The mean body mass index was slightly higher in females when compared to males (p>0.05). No significant differences were found in serum creatinine level, e-GFR, CPB period, period of hospitalization, and period of ICU between the pre-op and post-op (p>0.05).

Table 2. *Clinical and demographic parameters of the study population.*

	Total Patient	Female	Male	p value
Sex (Female/ Male)	n:46	n:8	n:38	
Age (Years)	61.08±9.41	66.25±13.55	60.0±7.86	p>0.05
BMI (kg/m^2)	27.55±1.50	28.22±1.01	27.40±0.05	p>0.05
Pre-op e-GFR (ml/min)	111.82±21.52	123.5±11.52	109.36±22.32	p>0.05
Post-op e-GFR (ml/min)	107.91±23.09	119.75±13.89	105.42±23.85	p>0.05
Serum Creatinine (mg/dL)	1.07±0.43	1.03±0.31	1.08±0.44	p>0.05
CPB Duration (min)	167.65±19.61	165.0±25.98	168.21±17.94	p>0.05
Duration of Hospitalization (Days)	3.78±0.65	4.0±0.70	3.73±0.63	p>0.05
Duration of ICU (Days)	1.56±0.64	1.25±0.43	1.63±0.66	p>0.05

The values are expressed as mean ± SE. Parametric comparisons were done using the student's t-test and non-parametric comparisons were done using the Mann-Whitney u test. BMI: Body mass index, e-GFR: Estimated glomerular filtration rate, CPB: Cardiopulmonary bypass, ICU: Intensive care unit.

In the comparisons between the pre-op and post-op 30 minute after CPB we evaluated the post-op level of IL-6 (7.15±2.08 ng/L) was found to be significantly higher than the pre-op (2.74±2.50 ng/L) (***p<0.0001) (Figure 2).

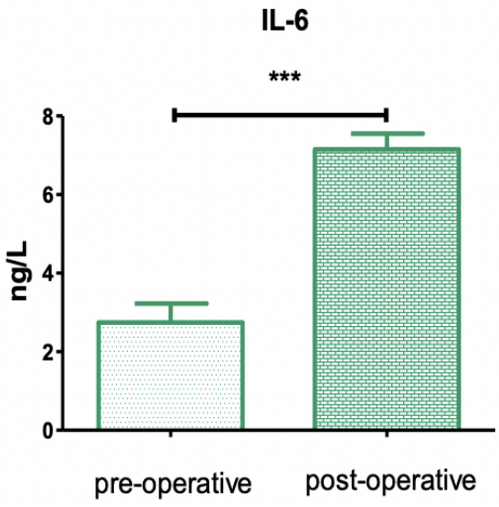

Figure 2. *The levels of plasma concentration of IL-6 during the pre-operative and the post-operative CPB (***p<0.0001).*

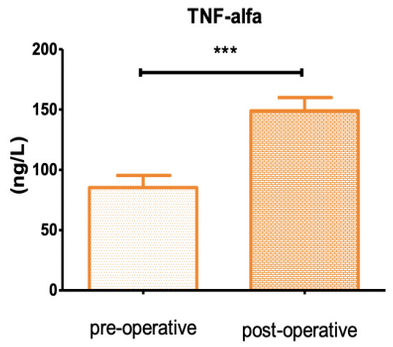

Figure 3. *The levels of plasma concentration of TNF-α during the pre-operative and the post-operative CPB (***p<0.0001).*

Likewise, TNF-α during the post-op (150.67±14.57 ng/L) was found to be significantly higher than the pre-op (83.63±9.33 ng/L) (***p<0.0001) (Figure 3). On the other hand, pre-op level of IL-1β (3.95±0.47 ng/L) significantly decreased during the post-op (0.38±2.04 ng/L and *p<0.05) (Figure 4).

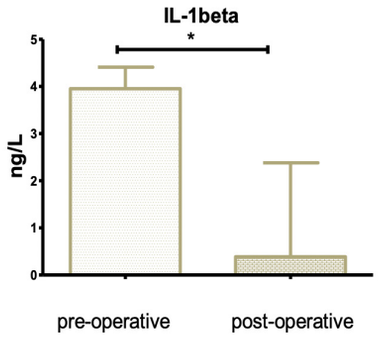

Figure 4. *The levels of plasma concentration of IL-1ß during the pre-operative and the post-operative CPB (*p<0.05).*

The mean ΔCt values of miR-21 expression levels in the pre-op (-6.55±7.11) and post-op (-11.78±6.98) periods of 46 patients who underwent Cardiopulmonary Bypass were compared, it was found that miR-21 expression significantly increased in the post-op (-11.78±6.98) (*p≤0.05) (Figure 5a).

When the mean ΔCt values of miR-10a expression levels in the pre-op (-12.22±3.55) and post-op (-11.60±3.36) periods were compared. This value was not found to be statistically significant (p>0.05) (Figure 5b).

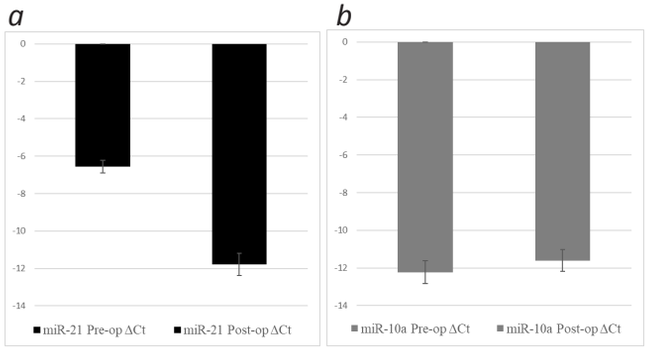

Figure 5.Expression *levels of pre-operative and post-operative total miR-21 mean ΔCt values (a) and expression levels of pre-operative and post-operative total miR-10a mean ΔCt values (b).*

When we compared the mean ΔΔCt values of miR-21 and miR-10a, downregulation was observed in the expression level of miR-10a (0.62±3.77) whilst the expression level of miR-21 was up-regulated (-5.22±7.25) (*p≤0.05) (Figure 6).

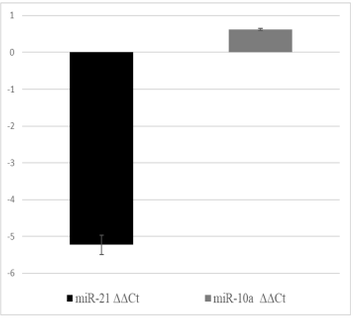

Figure 6. *Difference in expression levels of total miR-21 and miR-10a mean ΔΔCt (PostΔCt-PreΔCt) values.*

After the comparison of the mean pre-op and post-op ΔC_T values of miR-21 and miR-10a, we calculated the fold change or relative quantification as $2^{-\Delta\Delta CT}$. No significant downregulation was observed in the expression level of miR-10a (4.11±6.81) whilst the expression level of miR-21 was excessively up-regulated (27981.70±101673.90) (*p≤0.05) (Figure 7).

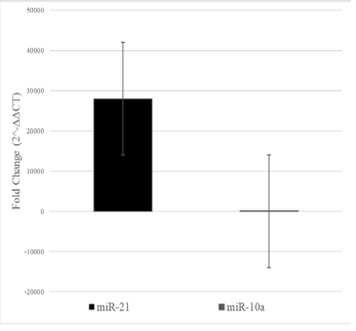

Figure 7. *The fold change and relative quantification (2 – ΔΔCT) of total miR-21 and miR-10a between pre-operative and post-operative (30 minutes after CPB) periods.*

After the full analysis of correlations between all the cytokines and miRNAs, those showing a significant (p<0.05) positive or negative correlation is given in Table 3. Pre-op IL-6 showed a high positive correlation with both post-op IL-6 and post-op IL-1β whereas it moderately correlated with pre-op miR-10a and showed low negative correlation with pre-op miR-21. None of the variables showed high correlation with eachother during the post-op period. Yet, post-op IL-6 showed low negative correlation with post-op miR-10a, low positive correlation with post-op miR-21, and moderate positive correlation with IL-1β. Also, post-op miR-21 showed moderate negative correlation with post-op miR-10a. Also, pre-op TNF-α exhibited a high positive correlation only with post-op TNF-α. Post-op TNF-α showed low negative correlation with post-op miR-21. Pre-op IL-1β was found to be negatively correlated with post-op TNF-α. Pre-op miR-21 showed a high positive correlation with post-op miR-21 and high negative correlation with pre-op miR-10a whereas it showed negative correlation with post-op miR-10a.

Table 3. *Pearson correlation between pre-operative and post-operative plasma concentrations of IL-6, TNF-α, IL-1β, and expression levels miR-21 and miR-10a. In each row, r represents correlation coefficient and p represents p value. Significant correlations are shown in bold.*

		IL-6 Pre-op	IL-6 Post-op	TNF-α Pre-op	TNF-α Post-op	IL-1β Pre-op	IL-1β Post-op	miR-21 Pre-op	miR-21 Post-op	miR-10a Pre-op	miR-10a Post-op
IL-6 Pre-op	r		0,742	0,126	-0,043	0,205	0,587	-0,202	-0,078	0,416	0,115
	p	1	**0,0001**	0,5957	0,8546	0,3844	**0,0065**	0,392	0,7423	0,0678	0,6281
IL-6 Post-op	r	0,742		-0,068	-0,093	0,293	0,407	-0,240	0,103	0,291	-0,113
	p	**0,0001**	1	0,7744	0,6951	0,2095	0,0747	0,3074	0,6648	0,2131	0,6337
TNF-α Pre-op	r	0,126	-0,068		0,705	-0,223	0,009	-0,147	-0,325	0,234	0,106
	p	0,5957	0,7744	1	**0,0005**	0,3444	0,9680	0,5340	0,1616	0,3201	0,6538
TNF-α Post-op	r	-0,043	-0,093	0,705		-0,447	-0,090	-0,233	-0,212	0,038	0,221
	p	0,8546	0,6951	**0,0005**	1	**0,0478**	0,7051	0,3213	0,3687	0,8713	0,3477
IL-1β Pre-op	r	0,205	0,293	-0,223	-0,447		0,387	0,052	0,085	0,030	0,218
	p	0,3844	0,2095	0,3444	**0,0478**	1	0,0909	0,8269	0,7192	0,8980	0,3555
IL-1β Post-op	r	0,587	0,407	0,009	-0,090	0,387		-0,043	0,037	0,077	0,214
	p	**0,0065**	0,0747	0,9680	0,7051	0,0909	1	0,8551	0,8761	0,7457	0,3634
miR-21 Pre-op	r	-0,202	-0,240	-0,147	-0,233	0,052	-0,043		0,523	-0,648	-0,451
	p	0,3929	0,3074	0,5340	0,3213	0,8269	0,8551	1	**0,0179**	**0,0019**	**0,0457**
miR-21 Post-op	r	-0,078	0,103	-0,325	-0,212	0,085	0,037	0,523		-0,186	-0,311
	p	0,7423	0,6648	0,1616	0,3687	0,7192	0,8761	**0,0179**	1	0,4308	0,1813
miR-10a Pre-op	r	0,416	0,291	0,234	0,038	0,030	0,077	-0,648	-0,186		0,163
	p	0,0678	0,2131	0,3201	0,8713	0,8980	0,7457	**0,0019**	0,4308	1	0,4899
miR-10a Post-op	r	0,115	-0,113	0,106	0,221	0,218	0,214	-0,451	-0,311	0,163	
	p	0,6281	0,6337	0,6538	0,3477	0,3555	0,3634	**0,0457**	0,1813	0,4899	1

4. DISCUSSION

Acute renal failure (ARF) prevalence is high among patients who undergo CPB (13). This condition can only be diagnosed via serum creatinine level (sCr) conventionally within 48 hours (14). Therefore, we need early novel diagnosis biomarkers to start preventive treatment of ARF before 48 hours (15). Inflammation mediated post-op consequences like ARF are caused by the contact of the blood with unknown surfaces (16). Recent studies point out a promising role for circulatory miRNAs in inflammation related unfavorable outcomes (17). Present study focused on inflammation induced cardio-renal biochemical pathway to quantify expression patterns of miR-21 and miR-10a during inflammatory response by IL-6, TNF-α, and IL-1β 30 minutes after CPB. Where the roles of miR-21 and miR-10a together or separately link to ARF in inflammatory pathway needs to be clarified. In our data, inflammatory IL-6 and TNF-α were found to be increased (***p<0.0001) shortly after CPB. Also, the ΔΔCT values (*p≤0.05) and fold change (*p≤0.05) manifested that post-op expression level of miR-21 was highly up-regulated. It has been reported that expression of miR-21 is excessively up-regulated and positively correlated with renal failure due to the increase of inflammatory IL-6 (18). Also, miR-21 expression was up-regulated in human renal epithelial cells when treated with different concentrations of TNF-α both in vitro and in vivo (19). Previous study done by X. Xu and A. J. Kriegel et. al. on the increase of IL-6 promoting excessive up-regulation of miR-21 demonstrated miR-21 as a damaging agent on renal tissue (9). IL-6, TNF-α are the most abundant inflammatory mediators in inflamed tissue (20). Thus, according to our data we demonstrated increased plasma levels of IL-6, TNF-α are primarily involved in inflammatory response 30 minutes after CPB. As we hypothesized, we have shown CPB-related inflammatory cytokines IL-6 and TNF-α excessively increase up-regulation of miR-21 shortly after CPB in vivo.

On the other hand, the post-op IL-1β was decreased (*p≤0.05). CPB is a complex operative application in which many cardio-renal biochemical pathways cross (21). The effect mechanism of cytokines is intracellular (22). Literature manifests that normally myostatin, also a cytokine, is over-expressed into the circulation by pathological heart tissue, it positively correlates with IL-1β increase. Previous study has shown that treatment of heart tissue cells with miR-21 mimic, prevented myostatin-induced increase of IL-1β (23). Our study supported the literature that there is a negative correlation between miR-21 and IL-1β concentration shortly after CPB. Thus, we propose the excessive up-regulation of miR-21 may have decreased the concentration level of IL-1β via over-expression of myostatin after CPB. Post-op miR-10a didn't change significantly and the fold change of miR-10a seems invisible compared to miR-21. However, the ΔΔCT values showed that miR-10a was down regulated (0.62±3.77) shortly after CPB. MiR-10a is mediated in renal tubules as the negative key regulator of cytokines (24). The intensity of down-regulation is mediated by TNF-α and IL-1β together (11). We think that the decrease in post-op IL-1β may cause low intensity of miR-10a down-regulation shortly after CPB. We hypothesized that after CPB, inflammation-related excessive release of miR-21 into circulation triggers down-regulation of miR-10a in renal tissue. Our data showed

that intensity of down-regulation of miR-10a has been suppressed due to the decrease of post-op IL-1β via CPB induced excessive expression of miR-21.

Additionally, despite not significant, miR-10a showed high positive correlation with IL-6 during pre-op period and low negative correlation during post-op period. The rate of IL-6 release depends on the down-regulation level of miR-10a (25). MiR-10a has been extensively reported on various tumor types (26). While miR-10a is positively correlated with advanced tumor, it negatively correlated with distant metastasis. It has been discussed that the heterogeneity of tumor cells may be the cause of different roles of miR-10a in tumor progression (27). We suggest that different correlation patterns of miR-10a and IL-6 in disease progression should be considered for future studies.

In addition, the release of IL-6, TNF-α, and IL-1β release during extracorporeal circulation like CPB is conflicting (28). There are many responsible possible reasons for such conflicting data (29). First is the mean period of CPB. In our study, the mean period of CPB was as twice longer than the previous study (30). Therefore, the longer contact of the blood with extracorporeal surfaces, the higher levels of primary cytokines shortly after CPB. Second, hypothermic CPB has been discussed to decrease some cytokines to a greater extent (31). Third, hemofiltration is being used to reduce the effects of inflammatory response during CPB (32). These factors may cause different levels of cytokine patterns after CPB. Hence, the contradictory release of IL-1β and TNF-α in the biochemical pathways after CPB needs clarification via further studies.

According to *Pearson* correlation coefficient analysis, no significant correlation has been found between cytokines and miRNAs after CPB. We quantified miR-21 and miR-10a based on total RNA. At this point we suggest that exosomal miR-21 and miR-10a may contribute a considerable amount of miRNA in the results (33). We believe that a significant correlation could be possible via expanding the data with exosomal miRNA isolation in the future studies. Previous findings on IL-6-induced excessive expression of miR-21 has been demonstrated as a damaging mechanism in renal tissue (34). Therefore, post-op miR-21 may be a reliable target in inflammation related renal tissue failure. Some studies manifested the protective role of miR-21 (35), whilst miR-21 is a comprehensively studied microRNA on renal disease progression (36). In one study done with nephropathy patients demonstrated glomerular filtration rate negatively correlated with urinary levels of miR-21. Up-regulation of urinary exosomal miR-21 has been showed to be a potential non-invasive biomarker for chronic kidney disease (37). Even though, back in 2008 Ronco et. al. addressed that heart and kidney dysfunction coexist as a result of systemic inflammatory pathway (38), Huang et. al. also recently discussed cardio-renal syndromes in relation with miR-21 (39). In the light of previous studies it has been demonstrated that miR-21 may cause renal failure. Underlying biochemical pathway needs to be clarified. Report by Glowacki et al. demonstrates that miR-21 has a central role and may represent a novel and predictive blood marker of kidney fibrosis (40). In that study, up-regulation of miR-21 was observed at day 4 with the disease progression. Also, their sample collection was based on renal tissue samples and serum. In our study, we quantified circulating high levels of miR-21 in plasma 30 minutes after CPB and shown that IL-6 and TNF-α trigger excessive up-regulation of miR-21. At the same time, we suggest that miR-21 induced post-op suppression of miR-10a pathway is also regulated via IL-1β. Hence, we suggest miR-21 and miR-10a to be non-invasive early diagnosis biomarker to detect acute renal failure 30 minutes after CPB. Our study includes supporting data for future studies.

There are some limitations to our study as the sample size was small. Therefore, larger cohort studies are needed. Moreover, due to Covid-19 restrictions we could not follow up the patients if they developed ARF after hospitalization. Findings of the present study is preliminary to illuminate the biochemical pathway between inflammatory cytokines, miR-21, and miR-10a on the way to ARF. We suggest this data to be considered for future studies. As we hypothesized, our study prospectively demonstrates for the first time that how post-CPB inflammation through IL-6 and TNF-α triggers excessive up-regulation of miR-21 and how suppression of miR-10a in this biochemical pathway being operated via miR-21 induced decrease of post-op IL-1β in vivo.

5. CONCLUSION

Prospectively, these data suggests that high miR-21 levels is a promising indicator and can be a candidate as an early novel biomarker for diagnosis of acute renal failure 30 minutes after CPB.

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