Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2017;42(3):436-445
DOI: 10.17826/cutf.323386

ARAŞTIRMA / RESEARCH

Role of cytokine gene polymorphisms on ramipril-altered inflammatory response and myocardial injury in patients undergoing coronary artery bypass surgery

Koroner arter bypass cerrahisinde ramiprilin miyokardiyal hasar ve inflamatuvar yanıttaki etkisi üzerinde sitokin gen polimorfizmlerinin rolü

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Cukurova Medical Journal 2017;42(3):436-445

Abstract

Purpose: Ramipril is effective in treating inflammatory myocardial injury by reducing cytokines such as TNF-α, IL-6, and IL-8. In this study, we investigated the effect of cytokine gene polymorphisms on inflammatory response which might be reduced by ramipril.

Material and Methods: Of 102 patients undergoing on-pump coronary artery bypass grafting surgery, 51 were the treatment group which received ramipril and the remaining 51 were the non-treated control group. TNF-α, IL-6, and IL-8 were measured with Enzyme-Linked Immuno Sorbent Assay (ELISA), before anesthesia induction (t1), at 20 min following cross clamping (t2), at the end of the operation (t3), and 24 hours after anesthesia (t4). Genotyping was performed with PCR method.

Results: While TNF-α increase began with surgery, IL-6 and IL-8 increase began with cardiac arrest during cardiopulmonary bypass and continued until the end of the operation. In contrast, only IL-8 remained high in the control group during the postoperative period, while TNF-α, IL-6, and IL-8 began to decrease. The decreases in IL-6 at t3 and in TNF-α at t4 were significant.

Conclusion: Ramipril might have a role in preventing inflammatory myocardial injury by reducing cytokine and TnT levels after cardiac arrest.

Key words: ACE inhibitors; cytokines, polymorphism.

Öz

Amaç: Ramipril, inflamatuar miyokardial hasarın tedavisinde TNF-α, IL-6 ve IL-8 gibi sitokin seviyelerini azaltarak etkili olmaktadır. Çalışmamızda, ramipril tedavisinin, koroner arter bypass cerrahisi esnasında oluşan inflamatuar yanıt ve miyokardiyal hasara yönelik etkisi üzerinde, sitokin gen polimorfizmlerinin herhangi bir rolü olup olmadığını araştırdık.

Gereç ve Yöntem: Çalışmamıza, koroner arter bypass cerrahisi uygulanan hastalardan cerrahi öncesi ramipril alan 51, kontrol grubu olarak ise ramipril almayan 51 olmak üzere toplam 102 hasta dahil edildi. Her iki grupta, anestezi başlamadan hemen önce (t1), kros klemp sonrası 20. dakikada (t2), ameliyatın sonunda (t3) ve anestezinin başlangıcından 24 saat sonra (t4) alınan kan örneklerinden TNFα, IL-6, IL-8 ve TnT serum düzeyleri ELISA yöntemi ile, genotipleme ise PCR-RFLP yöntemleri ile belirlendi.

Bulgular: Her iki grupta TNF-α artışı cerrahi ile başlarken, IL-6 ve IL-8 artışı kardiyopulmoner bypass sırasında kardiyak arrestle başlayıp ameliyat sonuna kadar devam etmiştir. Postoperatif dönemde ise sadece IL-8 kontrol grubunda yüksek kalırken; çalışma grubunda TNF-α, IL-6, IL-8 düşüşe geçmektedir. t3 zaman aralığında IL-6, t4 zamanında TNFα değerlerinde anlamlı azalma gözlenmiştir.

Sonuç: Ramiprilin kardiyak arrest sonrası sitokin ve TnT düzeylerini azaltarak inflamatuar miyokardiyal hasarı önlemede yeri olabilir.

Anahtar kelimeler: ACE inhibitörleri, sitokin, polimorfizm.

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Geliş tarihi/Received: 20.08.2016 Kabul tarihi/Accepted: 23.09.2016

INTRODUCTION

Angiotensin converting enzyme (ACE) activity, which increases during cardiopulmonary bypass (CPB), contributes to oxidoinflammatory injury by increasing superoxide anions, cytokines, leukocyte chemo-attractants, adhesion molecules, interleukins, and C-reactive proteins. This injury may result in organ dysfunction and/or failure, especially to the lungs, kidneys, and heart[1,2]. Oxidoinflammatory injury developing during CPB was shown to improve with pharmaceutical interventions such as corticosteroids, statins, and ACE inhibitors (ACE-I)[3]. Reducing the level of proinflammatory cytokines such as TNF-α, IL-6 and chemotactic cytokines (such as IL-8) may result in a positive outcome[3,4]. An ACE-I, ramipril is known to be effective in reducing mortality and morbidity in hypertension, left ventricle dysfunction, congestive heart failure, coronary artery disease, myocardial infarction, diabetic peripheral artery disease, and graft failure. It also alleviates oxidoinflammatory injury[5,6]. Despite the positive effects of ramipril, different results may occur as a result of gene polymorphism of ACE and cytokines, as well as the demographic characteristics of the patients. Consistent results cannot be replicated in meta-analysis and studies about ACE gene polymorphism[7,8]. Although ramipril was shown to reduce oxidoinflammatory myocardial injury in on-pump CABG in our previous study, ACE gene polymorphism was discovered not to be effective in this case[9]. The genetic structure of the individuals also affects the cytokine plasma levels stimulated through cardiac operations[10-13]. It was suggested that the alteration in proinflammatory cytokines (IL-6, IL-8, TNF-α) which appear during CPB could have arisen from IL-6 G-174C (*rs*1800795), IL-8 T-251A (rs4073) and TNF-α G-308A (rs1800629) polymorphisms[7,8,14-17]. Therefore, we aimed to investigate the influence of IL-6 -174 G/C, IL-8 -251 T/A and TNF-α -308 G/A polymorphisms on the ramipril-treated oxidoinflammatory subclinical myocardial injury in on-pump CABG.

MATERIAL AND METHODS

Study protocol

This study was approved by the ethics committee of our institution (BAP-TF CTB (NS) 2009-3), and informed consent was obtained from all patients. The study and control groups had similar properties with the exception of ACE-I treatment. All subjects were selected among patients followed up at our Cardiovascular Surgery Clinic, and all patients were handled by the same surgery, anesthesia, and care teams. The treatment group consisted of 51 patients who were scheduled for a coronary artery bypass surgery and who were on ramipril (2,5 mg/day) therapy for at least 7 days, while 51 patients who did not receive ramipril or any other ACE-Is constituted the control group. Table 1 summarizes the demographic and surgical data of these patients. Patients with unstable angina pectoris, acute MI, EF<40%, and respiratory, hepatic, and renal disorders were not included in the study. All cardiac medications were continued until the day before surgery. After the completion of the CABG, the patients were transferred to the intensive care unit. Postoperative care was standardized for all patients, and extubation was done as early as possible.

Cardiopulmonary Bypass

All patients were given 1 mg alprazolam orally (Xanax, Eczacıbaşı, Turkey) for sedation the night before surgery. Following neuroleptic anesthesia, a standard median sternotomy was performed, and a two-stage venous and aortic cannulation and continuous-flow CPB with moderate hypothermia were initiated.

After cross-clamping (CC) the aorta, blood cardioplegic solution was administered antegradely. The left internal thoracic artery was the standard conduit for all left anterior descending (LAD) arteries, while all others were revascularized by using the greater saphenous veins. Aortic clamps were removed after the completion of the distal anastomoses, and the proximal anastomoses were performed after the application of a side clamp, after the resumption of heartbeat.

Blood sampling

Arterial blood samples were collected before the induction of anesthesia (t1), 20 min after cross-clamping (t2), at the end of surgery (t3), and 24 h after the start of anesthesia (t4).

Table 1. Demographic and clinical features of study grups

	ACE-I	Control (No-ACE-I)	p
n (%)	51	51	
Male/Female	38/13	35/16	0.078
Age (years)	67±2	69±3	0.441
Body mass index	26.4	27.8	0.849
Current smoker	42 (82%)	39 (76%)	0.078
Treated hypertension	35 (68%)	32 (62%)	0.744
Diabetes Mellitus	14 (27%)	16 (31%)	0.986
Dyslipidemia	15 (29%)	14(27%)	0.429
CBP time (min)	85.50 (65-127)	82.50 (60-123)	0.367
Cross clamp time (minute)	57.50 (45-84)	52.;2 (30-88)	0.227
Stroke	3(5.88%)	2 (3.92%)	>0.05
Peripheral arterial disease	4(7.84%)	5(9.8%)	>0.05
CoronaryArtery by pass	3.2±0.4	3.4± 0.2	>0.05

Mean: average, Abbreviations: ACE-I Angiotensin converting enzyme inhibitor; *Continuous variables (mean±std dev or median (min–max)); † Categorical variable (%); ‡ Significant at $p<0.05$

Biochemical assay

Measurement of TNF-α, IL-6, and IL-8

The TNF-α, IL-6, and IL-8 concentrations were studied with a quantitative sandwich enzyme immunoassay (ELISA) from each blood sample taken at the different time intervals. The commercial kits used for this purpose were purchased from the DIAsource EASIA Kit (DiaSource Assays SA, Belgium).

Measurement of troponin T (TnT)

The same blood samples were also used for the establishment of troponin T levels as a marker of myocardial damage during CPB surgery[18] by the electrochemiluminescence method.

Determination of polymorphisms

Genotyping

In order to genotype the subjects, the standard salting-out Proteinase K method was used to extract the genomic DNA[19]. Genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Polymorphism analyses of the TNF-α -308 G/A[20], IL6 -174 G/C[21] and IL-8 -251 A/T[22] regions were carried out according to the analyses previously described with minor modifications as described below.

TNF-α -308 G/A

TNF-α -308 G/A was detected by using amplification of a 107 bp region using the primers 5'-AGGCAATAGGTT TTGAGGGCCAT-3' (forward) and 5'-TCC TCC CTG CTC CGA TTCCG-3' (reverse). Amplification reactions were carried out with 50 ng genomic DNA in a total volume of 25 µl, containing 10XPCR buffer, 1.5 mM $MgCl_2$, 100 µmol dNTP mixture, 50 pmol of each primer, and 1 U Taq polymerase. The reaction was incubated for a single cycle of 1 min at 95 °C, followed by 35 cycles of 1 min 94 °C, 1 min 57 °C and 1 min 72 °C, and a final extension of a single cycle at 72 °C for 5 min. PCR was carried out in a thermal cycler (Applied Biosystems). To detect -308 G/A variant alleles the PCR product was digested with *Nco*I at 37°C overnight, by leaving a 107 bp fragment when the variant was present and products of 87 and 20 bp fragments when the normal allele was present, and visualized on 3% agarose gel[20].

IL-6 -174 G/C

A 431-bp region of the IL-6 gene promoter containing the site of the polymorphism was amplified by PCR using the primers 5'CAGAAGAACTCAGATGACTG3' and 5'GTGGGGCTGATTGGAAACC3' as described previously[21]. Amplification reactions were carried out with 50 ng genomic DNA in a total volume of 25 µl, containing 10XPCR buffer, 1.5 mM $MgCl_2$, 100 µmol dNTP mixture, 50 pmol of each primer and 0.5 U Taq polymerase. The reaction was incubated for a single cycle of 1 min at 95 °C, followed by 35 cycles of 1 min 94 °C, 1 min 59 °C and 1 min 72 °C, and a final extension of a single cycle at 72 °C for 5 min. PCR was carried out in a thermal cycler (Applied Biosystems).

PCR products were digested with 5 units of NlaIII restriction endonuclease at 37 °C. Digested fragments were electrophoresed in a 1% (wt/vol) agarose gel and bands were visualized under UV after ethidium bromide staining. The product was electrophoresed in a 1% (wt/vol) agarose gel and bands were visualized under UV after ethidium bromide staining. For the -174G/C polymorphism, three different genotypes were determined which are G/G 229 bp, 173 bp, and 29 bp; G/C 229 bp, 173 bp, and 122 bp; C/C 229 bp, 122 bp, 51 bp, and 29 bp[21].

IL-8 -251 A/T

The polymorphism IL-8 -251 A/T was detected by PCR using the primer pair 5'-CCATCATGATAGCATCTGTA -3' and 5'-CCACAATTTGGTGAATTATTAA-3' [22]. Amplification reactions were carried out with 50 ng genomic DNA in a total volume of 25 µl, containing 10XPCR buffer, 1.5 mM $MgCl_2$, 100 µmol dNTP mixture, 50 pmol of each primer and 0.5 U Taq polymerase. The reaction was incubated for a single cycle of 1 min at 95 °C, followed by 35 cycles of 1 min 94 °C, 1 min 55 °C and 1 min 72 °C, and a final extension of a single cycle at 72 °C for 5 min. PCR was carried out in a thermal cycler (Applied Biosystems). After PCR, the product, a 349-bp fragment, was digested with MunI restriction enzyme (MBI Fermentas, Canada) overnight at 37 °C and then separated by electrophoresis in 2% agarose gel stained with ethidium bromide. The digestion fragments had 349 bp with TT genotype, 202 and 147 bp with AA genotype, and 349, 202 and 147 bp with AT genotype[23].

Statistical analysis

A pilot study was performed to determine the minimum required sample size. Minimum sample size was determined as 47 in each group based on a 30% change in TNF-alpha scores between patient and control groups (α=0.05, 1-β=0.80). Individual-level univariate analyses were performed to compare the baseline characteristics. Normality assumption was checked by using the Shapiro-Wilk test. To compare the two implementation groups for continuous variables, and for normally and non-normally distributed data, Student's *t* test and the Mann-Whitney *U* test were used. Relationships between categorical variables were tested by *chi*-squared test. Next, repeated measure of ANOVA models was used for detecting associations between the cytokine and treatment groups. The mean and standard deviations and percentages were given as descriptive statistics. All analyses were performed in SPSS for Windows version 22.0. A two-sided *p* value < 0.05 was defined as statistically significant.

RESULTS

The demographic features, clinical characteristics, and intraoperative data did not differ significantly between the two groups (Table 1). We did not observe any significant side effects of ramipril during the preoperative, perioperative, and postoperative periods, and none of the patients received prolonged intensive care. All patients were transferred from the intensive care unit to a hospital ward by the third postoperative day, at the latest.

The TNF-α levels had a tendency to increase from t1 to t2 and t2 to t3 in both groups. They had fallen to the starting values by the end of 24 h from the commencement of the operations. The difference between t2–t3 and t3–t4 in the control group was found to be statistically significant; however, in the treatment group, the drop between t3 and t4 was significant (p<0.05). In the control group, the patients' t3 and t4 values in comparison with the basic parameters remained high. For the treatment group patients, this was noted to be high during t3 and approximated to the basic levels in t4. This difference in terms of declination was found to be significant for the TNF-α in those patients who received ramipril for the t4 time interval (p<0.05) (Figure 1). The IL-6 levels were found to be steady and close to the basic values until cross-clamping in the control group patients and inclined upward from the t2 interval, reaching significance at t3, and finally dropping at the t4 level. The difference in this drop was found to be significant (p<0.05).

As for the treatment group, the values were almost the same, showing significance only between t2 and t3, with the disappearance of this significance at t4. The t3 and t4 values in both groups remained significant when compared with the basic values (p<0.05). When both groups were compared with each other, there was a significant difference between them at the t3 interval levels (p<0.05), which disappeared at t4 (Figure 1).

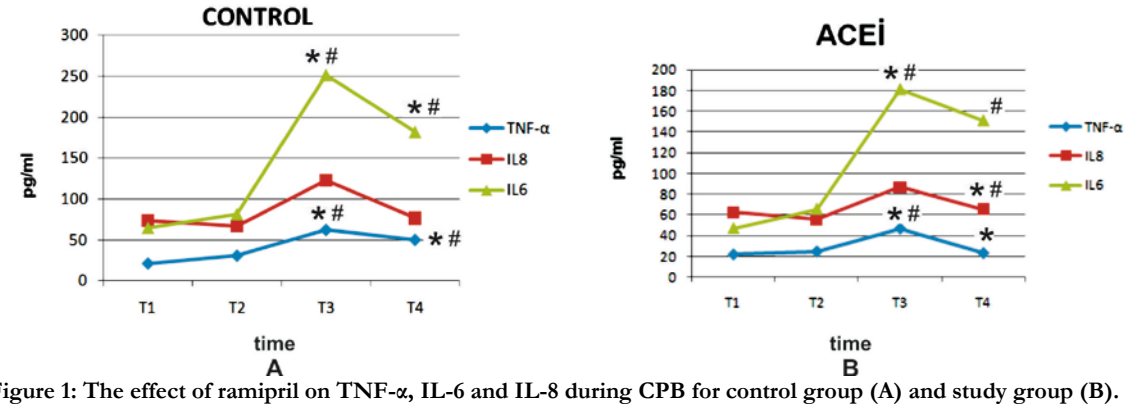

Figure 1: The effect of ramipril on TNF-α, IL-6 and IL-8 during CPB for control group (A) and study group (B).

The results are given as the mean [95% CI]. *P<0.05 denotes statistical differences from the previous time interval value, # P<0.05 denotes statistical differences from the base value.
Mean: average, ACE-I Angiotensin converting enzyme inhibitor, TNF-α Tumor necrosis factor alpha, IL-6 Interleukin-6, IL-8 Interleukin-8

Table 2. The distributions of the TNF-α, IL-6 ve IL-8 polymorphisms between the groups

	ACE-I (n=51)	Control Group (No-ACE-I) (n=51)	p
TNF α -305 G/A			
GG (n/%)	26 (50.98%)	30 (58.82%)	0.680
AG (n/%)	12 (23.53%)	12 (23.53%)	
AA (n/%)	13 (25.49%)	9 (17.65%)	
IL-6 -174 G/C			
GG (n/%)	10 (19.61%)	10(19.61%)	0.052
GC (n/%)	40 (78.43%)	33(64.71%)	
CC (n/%)	1 (1.96%)	8(15.68%)	
IL-8 -251 A/T			
AA (n/%)	9 (17.65%)	10(19.61%)	0.935
AT (n/%)	22(43.14%)	21 (41.18%)	
TT (n/%)	20 (39.21%)	20 (39.21%)	

ACE-I Angiotensin converting enzyme inhibitor, TNF-α Tumor necrosis factor alpha, IL-6 Interleukin-6, IL-8 Interleukin-8

The IL-8 levels displayed a similar pattern toward t2 in both groups and started to increase after the t2 time interval in the control group during t3 to t4, whereas it showed a downward trend in the treatment group during the same time interval. The increase in the IL-8 levels in the control group throughout t2 to t3 showed borderline significance (p=0.0528).

The t4 levels in the treatment group were found to be significantly low when compared with the t3 and base values (p<0.05) (Figure 1). The TnT levels in both groups showed an increase only in the t3 interval when compared with the previous interval values. This was found to be significant in both groups (p<0.05). The values obtained at the t3 and t4 intervals in both groups showed a significance when compared with the base values (p<0.05). Time-related changes in the TnT values between the groups had no significant difference.

The effects of TNF-α -308 G/A, IL-6 -174 G/C and IL-8 -251 A/T polymorphisms on cytokines and TnT were as follows; The distributions of genotypes of the TNF-α -308 G/A, IL-6 -174 G/C, and IL-8 -251 A/T polymorphisms (Table 2), together with the serum levels of IL-6, IL-8, TNF-α, and TnT measured in the four different time intervals, were compared between groups. The results are given in tables 3 and 4. No significant differences were found between groups in terms of distributions of genotypes of the TNF-α -308 G/A, IL-6 -174 G/C, and IL-8 -251 A/T polymorphisms and the serum levels of IL-6, IL-8, and TNF-α (Table 2 and Table 3) and TnT (p>0.05) (Table 4).

Table 3. Effects of TNF-α-308 A/G, IL-6-174 G/C, IL-8-251 T/A genotypes on the serum levels of TNFα, IL-6 and IL-8 for control and experiment groups.

Variables*†	ACE-I (n=51)				Control Group (No-ACE-I) (n=51)			
TNF α -305 G/A	**GG (26)**	**AG (12)**	**AA (13)**	**P**	**GG (30)**	**AG (12)**	**AA (9)**	**P**
TNF α (pg/ml) time 1	19.77 ± 13.50	33.55 ± 45.00	19.21 ± 12.65	0.709	21.61 ± 13.95	23.52 ± 16.65	17.99 ± 16.74	0.614
time 2	25.01 ± 16.64	24.99 ± 24.90	19.03 ± 13.97	0.423	34.39 ±51.88	29.96 ± 16.65	20.12 ± 15.02	0.323
time 3	48.39 ± 50.80	48.52 ± 59.29	40.70 ± 28.34	0.883	61.65 ±72.86	70.86 ± 46.29	50.86 ± 52.35	0.188
time 4	23.46 ± 18.68	24.72 ± 18.03	24.38 ± 18.17	0.976	36.50 ±44.01	51.60 ± 71.45	90.62 ± 200.79	0.574
IL-6 -174 G/C	**GG (10)**	**GC (40)**	**CC (1)**		**GG (10)**	**GC (33)**	**CC (8)**	
IL-6 (pg/ml) time 1	25.92 ±24.06	52.32 ±81.77	41.70	0.733	29.43 ±27.74	87.29 ±131.37	19.26 ±7.90	0.227
time 2	78.62 ± 114.78	63.42 ± 75.93	19.00	0.689	48.75 ± 76.81	101.07 ± 162.69	38.35 ± 18.01	0.435
time 3	114.70 ± 155.19	189.56 ± 149.99	468.00	0.167	264.86 ± 188.55	237.37 ± 159.55	304.38 ± 167.97	0.520
time 4	85.42 ± 67.76	168.89 ± 148.74	NC	0.097	236.09 ± 117.64	170.75 ± 128.34	156.27 ± 130.71	0.239
IL-8 -251 A/T	**AA(9)**	**AT (22)**	**TT (20)**		**AA(10)**	**AT (21)**	**TT (20)**	
IL-8 (pg/ml) time 1	28.18 ±40.25	52.10± 61.64	84.46±1 31.00	0.133	89.17 ±145.57	83.81 ±158.46	58.46 ±57.59	0.574
time 2	40.18±69. 33	59.39 ±66.30	53.61±6 4.57	0.216	100.77 ±198.50	75.38 ±115.92	41.14 ±26.74	0.491
time 3	45.86±29. 66	46.29±7 1.75	148.74± 236.01	0.078	149.85 ±194.06	147.06 220.46	98.17 ±150.07	0.779
time 4	66.43 ±103.10	50.05 ±72.86	78.69±1 14.42	0.340	107.78 ±111.01	57.98 ±48.52	81.96 ± 156.68	0.693

Mean: average, ACE-I Angiotensin converting enzyme inhibitor, TNF-α Tumor necrosis factor alpha, IL-6 Interleukin-6, IL-8 Interleukin-8, *Continuous variables (mean±std. dev), †Significant at $p<0.05$

DISCUSSION

Ischemia, ischemia-reperfusion, and increase in perioperative proinflammatory cytokines are the main reason behind emergence of myocardial injury which is a serious complication of cardiopulmonary bypass[24,25]. Proinflammatory cytokines (e.g. TNF-α and IL-6) and chemotactic cytokines (e.g. IL-8) released due to increased ACE during on-pump CABG may lead to or increase subclinical inflammatory myocardial injury by activating polymorphonuclear cells, macrophages, neutrophils, and eosinophils[26]. This negative effect may be explained by approximately 90% of ACEs having a role in local synthesis of Angiotensin II existing in the myocardium and arterial system.

The remaining 10% of circulating ACE is mostly responsible for the acute changes in blood pressure[27]. In our study, values of TnT increased in both groups with CC and tended to decline during the postoperative period and did not lead to myocardial dysfunction reaching significantly pathological levels. However, the lower TnT levels in the ramipril group suggest the importance of myocardial ACE. Increases of TNF-α**,** IL-6, and IL-8 in blood and myocardium during and after CPB were detected in several studies[28,29].

In our treatment and control groups, although TNF-α elevation began with the operation, IL-6 and IL-8 elevation began with putting CC in CPB, and continued until the end of the operation. Only IL-8 remained high in the control group postoperative, while TNF-α, IL-6, IL-8 declined in the treatment group. TNF-α was shown to lead to myocardial necrosis and dysfunction by impairing Ca-metabolism and cell membrane integrity in

myocardial infarction, leading to heart failure and myocardial dysfunction which may develop after cardiac surgery[30,31]. TNF-α-activated nuclear factor kB (NFkB) may aggravate the present injury by initiating the synthesis of the other proinflammatory cytokines such as IL-6 and IL-8[31] . While IL-6 increases in myocardial ischemia and ischemia reperfusion, it may lead to ventricular dysfunction. IL-6 increase affects the course of the disease and recovery process in patients experiencing acute MI[32]. IL-8 was shown to lead to neutrophil accumulation in myocardial tissue of reperfused rabbit, dog, and human hearts[33,35]. Superoxide anions and hydrogen peroxides which arise from neutrophils activated by newly synthesized IL-8 may increase myocardial injury[36]. We may state that TNF-α, which began to increase with the commencement of the operation in both treatment and control groups, may lead to a very mild myocardial injury with the TNF-α levels until CC is put. TnT values significantly increased over baseline values after CC was put.

Table 4. Effects of Tnf-A-308 G/A, Il-6-174 G/C, Il-8-251 T/A genotypes on the serum levels of tnt for control and study groups

Variables*†	**ACE-I (n=51)**				**Control Group (No-ACE-I) (n=51)**			
TNF α -305 G/A	GG (26)	AG (12)	AA (13)	p value	GG (30)	AG (12)	AA (9)	P value
TnT(pg/ml) time 1	0.03±0.06	0.01± 0.01	0.006 ± 0.008	0.444	0.01±0.004	0.01±0.006	0.01± 0.005	0.960
time 2	0.38± 0.81	0.13± 0.23	0.13± 0.18	0.451	0.27 ±0.39	0.25±0.30	0.26± 0.37	0.836
time 3	0.78± 2.07	1.06± 2.66	1.96± 5.59	0.129	0.72±0.42	0.71±0.44	0.62± 0.39	0.624
time 4	1.05± 2.25	0.58± 0.66	1.58± 4.20	0.568	0.83 ±0.85	0.94±0.99	0.57± 0.68	0.564
IL-6 -174 G/C	GG (10)	GC (40)	CC (1)		GG (10)	GC (33)	CC (8)	
TnT(pg/ml) time 1	0.01±0.01	0.01±0.02	0.001	0.260	0.01±0.004	0.01±0 .005	0.009±.004	0.713
time 2	0.10±0.18	0.25±0.51	0.25	0.346	0.23±.34	0.29±0.38	0.18±0.35	0.391
time 3	0.26±0.23	1.84± 4.84	21.31	0.078	0.57±.40	0.74±0.41	0.67±0.48	0.160
time 4	0.25±0.24	1.48± 3.60	16.10	0.109	0.66±.70	0.84±0.91	0.87± 0.84	0.750
IL-8 -251 A/T	AA (9)	AT (22)	TT (20)		AA (10)	AT (21)	TT (20)	
TnT(pg/ml) time 1	0.003±0.003	0.007±0.008	0.016± 0.03	0.158	0.01± .003	0.01±0.004	0.01±0.006	0.256
time 2	0.42±0.63	0.217±0.546	0.139± 0.21	0.147	0.33± 0.43	0.27 ±0 .09	0.26±0.37	0.697
time 3	2.90±6.93	2.46±6.17	0.804± 2.01	0.627	0.66±0.42	0.73 ±0.39	0.70±0.47	0.823
time 4	2.08±5.26	2.17±4.71	0.514± 0.82	0.961	0.72±0 .71	0.88±1.00	0.83±0.83	0.915

Mean: average, ACE-I Angiotensin converting enzyme inhibitor, TNF-α Tumor necrosis factor alpha, IL-6 Interleukin-6, IL-8 Interleukin-8, TnT troponin T; *****Continuous variables (mean±std. dev); **†**Significant at $p<0.05$

The increased myocardial injury values may be explained with continuing elevation of TNF-α until the end of the operation after CC is put or triggering of TNF-α the other cytokines or independent increases of IL-6 and IL-8 values. The time when TnT values reaches a peak is the time when the operation has ended, and all three cytokines reach their peak values. TnT values begin to decline during the postoperative period by the tendency of cytokines to decline. The similar course of cytokines and TnT is important for showing the association between cytokines and myocardial injury. However, the declining tendency of TnT values after t3 being similar with IL-8 rather than TNF-α and IL-6 may be explained by oxidative damage caused by neutrophils accumulating in the myocardium due to IL-8[36] . No clinical findings of inflammatory myocardial injury were encountered in our patients as they had little co-morbid factors and their ventricular functions were good. TNF-α, IL-6, IL-8,

and TnT levels in the control and treatment groups may be explained with cardiac ischemia developing in the course of CPB and ischemia reperfusion coming after. This oxidoinflammatory process may result in complications and even death in patients whose ventricular function is impaired and/or at risk. ACE inhibitors were shown to reduce myocardial injury and cardiovascular events, and prolong survival in the studies conducted with the CAD patients who were treated with invasive and noninvasive methods[5,37,38]. Protective effects of these drugs on endothelium size and function, induction of angiogenesis, and reduction of tissue inflammation during preventing atherosclerotic plaque, are also effective in cardioprotective as well as antihypertensive functions.

The therapeutic and protective effects of ramipril are recognized in all subgroups of the cardiovascular system[2,6]. Although the demographic characteristics and operative details of both groups were similar, TNF-α remained lower after beginning of the operation in the treatment group, IL-6 and IL-8 showed a similar course with the start of CPB, and TnT levels were consistent with the cytokines, supporting the protective effect of ramipril against inflammatory myocardial injury.

We found that cytokine gene polymorphisms have no role on the effect of ramipril. The differences between cytokines due to genetic differences among the individuals may negatively affect postoperative mortality and morbidity even if they have similar demographic characteristics[16,18]. Cytokine production shows significant differences among individuals due to genetic factors. These differences were put forward to be related with cytokine gene polymorphism and varied between populations[7,8,10,11] . Therefore, conflicting results have been obtained from those studies designed for investigating the associations between cytokine levels and the polymorphisms of the cytokine genes[10-13,39-42] . In our study, we could not find any association among IL-6, IL-8, or TNF-α in the preoperative, intraoperative or postoperative periods of CABG and IL-6 -174 G/C, IL-8 -251 A/T, and TNF-α -308 G/A polymorphisms, respectively, in patients who were receiving ramipril. Consistent with the results of our study, Galinanes et al. proposed that TNF-α -308 G/A polymorphism did not affect preoperative or postoperative TNF-α plasma levels[39] . Westerberg et al. stated that they could find no evidence for the relationship between post-CPB TNF-α levels and TNF-α gene polymorphism[40]. On the other hand, Yoon et al. found that TNF-α levels elevated during and after CPB in individuals who had TNF-α -308 GA/AA genotype (n=25) compared to the TNF-α -308 GG group (n=225)[10]. Bittar et al. showed an association between TNF-α -308 AA genotype and postoperative elevated TNF-α levels[10].

Wang et al. found that postoperative IL-6 and IL-8 levels were significantly higher in patients who were carrying IL-6 -174 GG and IL-8 -251 AA genotypes[13] . However, there was no association between TNF-α level and TNF-α -308 G/A polymorphism in their study investigating the association between IL-6, IL-8, and TNF-α levels and IL-6 -174 G/C, IL-8 -251 A/T, and TNF-α -308 G/A polymorphisms after off-pump CABG. Burzotta et al. and Gaudino et al. found an association between IL-6 GG genotype and elevated IL-6 levels in the postoperative period (higher than C allele carriers after coronary surgery)[41,42] yet Yoon et al. have not observed such an association[11].

In general, different results were obtained in those studies investigating the relationship between cytokine gene polymorphisms and cytokine levels before and after cardiac surgery. In our study we observed that cytokine levels were not affected by cytokine gene polymorphism which did not reflect to myocardial injury.

We may conclude that ramipril has a role in prevention of inflammatory myocardial injury by reducing cytokine and TnT levels after cardiac arrest. However, TNF-α, IL-8 and IL-6 gene polymorphisms had no effect on the outcome.

Acknowledgments

This work has been supported by grant No. FEFBAP/2009-0002 from the Adıyaman University Scientific Projects Unit (ADYUBAP).

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