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Interleukin-8 polymorphism has no effect on levels of IL-8 following coronary artery bypass grafting

Atike Tekeli[1], C. Selim İsbir[2]*, Arzu Ergen[1], Nilüfer Bozkurt[1], Uzay Görmüş[1], Sibel Bulgurcuoğlu[1], Sinan Arsan[2]

[1]Department of Molecular Medicine, The Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey

[2]Department of Cardiovascular Surgery, Medical Faculty, Marmara University, Istanbul, Turkey

Objectives: Cardiopulmonary bypass (CPB) induces a whole body inflammatory response and organ dysfunction eventually. This acute phase response after cardiac surgery induces the release of proinflammatory and antiinflammatory cytokines mainly interleukin (IL)-6, TNFα, IL-1β, IL-8 and IL-10. The aim of our study is to investigate whether IL8 polymorphism effect the plasma levels of IL-8 and also whether the levels of IL-8 have an effect on the outcome of the patients following surgery.

Methods: Fourty seven patients underwent elective coronary artery bypass grafting (CABG) procedure with CPB. Genotyping for IL-8 (C1633T) was performed using a polymerase chain reaction-restriction fragment length polymorphism technique. Concentrations of IL-8 were measured before the induction, 4, 24 and 72 hours after the surgery by enzyme-linked immunosorbent assay.

Results: IL8 levels although increased following CABG, did not show any significant difference between the carriers of 1633C and 1633T alleles at any time ($p>0.05$). Elevated IL8 levels at 24th postoperative hour were shown to be related with higher lactate levels, inotropic requirements in high doses and longer ventilation and ICU stay times. ($p=0.000$, $p=0.001$, $p=0.001$ and $p=0.012$ respectively).

Conclusion: These data suggest that IL-8 levels increase after CPB and associated with dreadfull postoperative course. IL-8 (C1633T) polymorphism however does not have any influence on the levels of IL-8.

Key words: Interleukin-8, gene polymorphism, coronary artery bypass grafting, cardiopulmonary bypass and systemic inflammatory response syndrome

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*Correspondence to: C. Selim İsbir, MD
Assoc Proffessor of Cardiovascular Surgery,
Marmara University Hospital,
Tophanelioglu Cad, No: 13-15,
Altunizade 80200 İstanbul, Turkey
Phone: +90 212 635 19 59
e-mail: isbir@yahoo.com
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Introduction

Cardiopulmonary bypass (CPB) provokes an inflammatory response, namely systemic inflammatory response syndrome (SIRS) leading to many important clinical implications postoperatively ranging from mild organ dysfunction to multiple organ failure with increased morbidity and mortality.[1] It is suggested in many experimental and clinical studies that this response is triggered by contact of blood elements with foreign surfaces meaning "contact activation", ischemia-reperfusion injury, endotoxemia and also trauma due to surgical procedure.[2,3] Heparinized blood contacting nonendothelial surfaces during extracorporeal circulation procedures activates monocytes, tissue macrophages, lymphocytes, platelets and endothelial cells. These cells when activated produce many proinflammatory and antiinflammatory cytokines such as IL-1β, IL-6, IL-8, tumor necrosis factor- alpha (TNF-α) and IL-10 mediating inflammatory processes following surgery.[4] Recently genetic polymorphisms of cytokines as IL-6, TNF-α has been shown to effect the levels of the proinflammatory cytokines and eventually lead to an enhanced SIRS following cardiac surgery.[5,6]

The gene encoding for IL-6 is located on chromosome 7p21[7] and IL-6 -174G>C polymorphism is found to be associated with higher plasma levels of IL-6 in acute inflammatory situations.[8] IL-8 is a member of chemokines which are superior family of proinflammatory proteins.[9] The gene encoding for IL-8 is located on chromosome 4q12.[10] IL-8 is also involved in inflammatory reactions however studies that are showing an association of the polymorphism of IL-8 with inflammation is mostly related with chronic diseases such as bronchial asthma, arthritis and bladder cancer.[10,11]

In this present study, our aim is to investigate whether the IL-6 and IL-8 polymorphisms effect the plasma levels of these two cytokines and also whether the levels of these cytokines have an effect on the outcome of the patients following coronary artery bypass grafting operation with CPB.

Materials and Methods

Patients

The present study was approved by the local ethics comittee and included 47 patients. 37 of the patients were male and the mean age was 56.28 ± 2.76 years. The patients with known inflammatory state such as fever, leukocytosis, arthritis, local or systemic infection, malignancy, immune system dysfunctions, unstable angina pectoris and patients on corticosteroid or other anti-inflammatory therapy except acetylsalysilic acid were excluded from the study.

Surgical procedure

All operations were performed in a standardized approach by a Jostra HL-20 roller pump (Jostra AG, Hirrlingen, Germany), membrane oxygenators (Jostra Quadrox, Hirrlingen, Germany), and a 40 μm arterial blood filter (Jostra AG, Hirrlingen, Germany). Mild to moderate (28 - 32°C) hypothermia and pulsatile flow of 2.2 to 2.4 L/m^2 were used. Myocardial protection was achieved with cold antegrade blood cardioplegia. Perfusion pressure was kept over 70 mmHg in all times. Aprotinin was not used in any of the patients. Induction and maintenance of general anesthesia with endotracheal intubation were standardized an all the patients (phentanyl, midazolam, pancuronium and isoflurane in oxygen with air).

IL-6 and IL-8 assay

Blood samples were drawn before surgery (t1) 4, 24 and 72 hours after CPB (t2, t3 and t4). These samples were immediately centrifuged (3000 rpm for 5 minutes) and plasma was seperated and frozen at -20°C until plasma IL-6 and IL-8 levels were determined by enzymelinked immunoassay (Cat No: KHC0061 and KHC0081 Biosource International, California, US).

Genotyping

DNA isolation Blood specimens were collected in tubes containing ethylenediamine-tetra acid (EDTA). DNA samples were extracted from whole blood with salting-out procedure.[12]

Analysis of IL-6-174 gene polymorphisms: IL-6-174 genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Briefly, 100 ng of genomic DNA was placed in 10 mmol/L TRIS-HCl, 50 mmol/L KCl buffer containing 0.2 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L for each primer and 0.5 U of Taq DNA polymerase in a total volume of 20 uL. Amplifications were done in an Applied Biosystems Gene Amp PCR system 9700 Gold Plate Thermocycler with the following process: 94°C for 5 minutes followed by 35 cycles at 94°C for 1 minute, 63°C for 30 seconds, and 72°C for 1 minute, and finally 72°C for 10 minutes. PCR products were digested with 1.5 U of Sfa NI restriction enzyme at 37°C overnight. The digested DNAs were separated on 2% agarose gel in 1x Tris borate EDTA buffer, followed by staining by an ethidium bromide solution. The IL-6-174 genotypes were typed by visualization under ultraviolet light. G allele results in 368 and 245 base pair fragments. C allele remains undigested.[7]

Analysis of IL-8 C1633T gene polymorphism: IL-8 C1633T genotyping was performed using a PCR-RFLP technique. Briefly, 100 ng of genomic DNA was placed in 10 mmol/L TRIS-HCl, 50 mmol/L KCl buffer containing 0.2 mmol/L of each dNTP, 1.5 mmol/L MgCl2, 0.25 mmol/L for each primer and 1 U of Taq DNA polymerase in a total volume of 20 uL Amplifications were done in a Applied Biosystems Gene Amp PCR system 9700 Gold Plate Thermocycler with the following process: 94°C for 5 minutes followed by 35 cycles at 94°C for 1 minute, 63°C for 30 seconds, and 72°C for 1 minute, and finally 72°C for 10 minutes. PCR products were digested with 1 U of Eco RI restriction enzyme at 37°C overnight. The restriction fragments were separated on 2% agarose gel in 1x Tris borate EDTA buffer, followed by staining by an ethidium bromide solution. The IL-8 C1633T genotypes were typed by visualization under ultraviolet light.[10]

Statistical analysis

Statistical analyses were performed using the SPSS version 11.00 for Windows (SPSS Inc. Chicago, IL, USA). Discrete variables were expressed as counts or percentages and compared with chi-square or Fisher's exact test when appropriate. Analysis of variance (ANOVA) test was used to compare concentrations of plasma IL-6 and IL-8 between individuals with different IL-6 and IL-8 genotypes and Bonferroni correction was performed. Continous variables were expressed as mean ± standard error of mean (SEM) and compared by student's t test or ANOVA for more than two groups. Results were considered statistically significant at $p<0.05$.

Results

The genotypic distribution and allelic frequencies of IL-6 and IL-8 were demonstrated in Table 1. Rare allele frequencies were 0.447 and 0.425 respectively for IL-174G>C and IL-8 C1633T genotypes. Allele frequencies were not significantly different from those reported previously.[10,13]

Baseline characteristics and perioperative details of the patients did not show any difference between the different genotypes of IL-6 and IL-8 (Tables 2 and 3). The 30-day mortality was 2.1% (n=1). Five patients required inotropic support perioperatively (4 patients needed 5-10 μ/kg/min for <24 hours and 1 patient needed >10 μ/kg/min for >24 hours) and only one required intraaortic baloon pump support.

Table 1
Distribution of IL-6 C174G and IL-8 C1633T genotypes and allelles

	Patients	(n: 47)
IL-6 C174G GENOTYPES	**n**	**%**
CC	9	19
GG	14	30
GC	24	51
ALLELLES		
C	42	44.7
G	52	55.3
IL-8 C1633T GENOTYPES		
CC	3	6.4
TT	10	21.3
CT	34	72.3
ALLELLES		
C	40	42.5
T	54	57.5

Table 2
Preoperative demographics of the patients.

	-174G>C				C1633T			
	GG (n=14)	GC (n=24)	CC (n=9)	p	TT (n=10)	TC (n=34)	CC (n=3)	p
Age (years)	56.5±3.95	55.08±2.58	59.1±2.54	0.344	54.3±2.76	55.6±2.20	69.6±3.38	0.138
Sex (male/females)	11/3	20/4	6/3	0.581	9/1	26/8	2/1	0.570
BSA (m^2)	1.79±0.05	1.78±0.03	1.76±0.07	0.730	1.72±0.06	1.80±0.03	1.77±0.05	0.484
Smoker	13	14	7	0.066	9	24	1	0.143
DM	6	7	4	0.592	1	13	3	0.016
HT	8	14	7	0.543	7	19	3	0.267
COPD	5	8	3	0.988	3	13	0	0.389
HL	11	13	8	0.098	7	22	3	0.449

BSA= body surface area, COPD= chronic obstructive pulmonary disease,CAD= coronary artery disease

Table 3
Perioperative data of the patients.

	-174G>C				C1633T			
	GG (n=14)	GC (n=24)	CC (n=9)	p	TT (n=10)	TC (n=34)	CC (n=3)	p
ACC time (min)	31±4.22	44±3.34	37.91±2.18	0.499	32.8±4.4	39.88±2.01	40.33±5.23	0.474
CPB time (min)	65±4.98	78.57±4.8	69.12±3.29	0.455	66±5.91	72.26±2.87	75.66±8.19	0.820
Number of grafts	2.77±0.40	3.5±0.22	2.87±0.20	0.412	2.7±0.30	3.11±0.18	3.33±033	0.484
Ventilation time (h)	6.1±1.01	9.07±1.47	7.2±3.08	0.420	5.6±1.05	7.97±0.72	9.3±1.2	0.465
Inotropic agent requirement (n)	1	2	2	0.847	1	3	1	0.417
IABP requirement (n)	0	1	0	0.300	0	1	0	0.823
ICU stay (h)	28.33±5.11	38.14±5.29	27.67±3.08	0.307	34.66±10.66	32.79±3.03	26.8±4.73	0.271
Hospital stay (d)	6.22±1.09	6.28±0.62	6.54±0.83	0.319	8.3±1.92	5..97±0.36	5±0.57	0.512

CPB=cardiopulmonary bypass, ACCtime= aortic cross clamp, ICU= intensive care unit

None of the patients suffered from postoperative bleeding or infections. The patients with different genotypes did not differ with respect to liver function tests (serum aspartate aminotransferase, serum alanine aminotransferase and serum bilirubin) and peak postoperative serum creatinine.

At baseline, mean IL-6 and IL-8 levels were independent of the genotype (6.33±0.41pg/ml for IL-6-174GG versus 6.52±0.18 pg/ml for -174C allele carriers, p=0.605 and 15.08±0.50 pg/ml for IL-81633 TT versus 15.57±0.82 pg/ml for 1633 C allele carriers, p=0.648). Serum levels of IL-6 increased significantly at 4th and 24th postoperative hours (50.39± 3.15 pg/ml and 159.30±6.18 pg/ml, respectively, p=0.000 compared with baseline levels). Ventilation time was slightly higher in the patients with elevated levels of IL-6 at 24th postoperative hour however this was not statistically significant (p=0.051). The increase in IL-6 levels was significantly genotype dependent (Mean IL-6 level at 4th postoperative hour was 58.95±3.33 pg/ml for -174C allele carriers versus 30.22±2.96 pg/ml for -174GG genotype, p=0.000 and mean IL-6 level at 24th postoperative hour was 178±4.65 pg/ml for -174C allele carriers versus 113.57±9.96 pg/ml for -174GG genotype, p=0.000). A similar response was observed for IL-8 with significantly increased levels at 4th, 24th and 72nd hours postoperatively (82.08±2.76 pg/ml, p=0.000, 57.54±2.46 pg/ml, p=0.000 and 49.65±1.83 pg/ml, p=0.000 compared with baseline levels respectively). Although the IL-8 levels were found to be elevated following surgery there was no significant effect of the IL-8 C1633T polymorphism on this elevation.

Eventhough the perioperative requirement of inotropes, ventilation time, ICU stay and 30-day

mortality were independent of the genotypes studied, these clinical conditions were found to be significantly associated with the elevated plasma concentrations of IL-8 at 4th, 24th and 72nd postoperative hours.

Discussion

Systemic inflammatory response following cardiac surgery is still a common and potentially devastating event associated with increased postoperative morbidity and mortality. Previous investigations designed to understand the mechanism of this complication mainly relied on the adverse effects of CPB such as activation of coagulation, complement, kallikrein and fibrinolytic systems.[14,15] Although the main cause of SIRS has been shown to be the extracorporeal circulation there are studies stating that other thoracic, abdominal and off-pump cardiac surgical procedures also lead to this complex event.[16-18] Proinflammatory cytokines, acute phase reactants as C-reactive protein and components of complement cascade as C5a are regarded as markers of immune response.[18]

It is evident that CPB induced SIRS is multifactorial and current data suggests several genetic polymorphisms of the proinflammatory cytokine genes affecting the extent of inflammatory response.[19] Brull and colleagues[20] were the first group to report that IL-6 gene promoter -174G>C and -572G>C polymorphisms influence the IL-6 response following CABG. In a study by Ryan and colleagues the carriers of TNF-β +252 AA genotype and IL-10 -1082G/A genotype were shown to have elevated lactate levels after cardiac surgery.[21] Previous studies have also demonstrated that IL-6 174G/C promoter polymorphism is likely to be associated with increased risk of coronary artery disease and higher systolic blood pressure in healthy men.[22,23] In another study, an association between -174C allele and higher levels of IL-6 was reported in patients with aortic aneurysms.[24]

The present study was designed to investigate whether genetic polymorphisms of IL-6 174G>C and IL-8 C1633T might influence the IL-6 and IL-8 levels in CABG patients. Our data supported the literature that CPB leads to an increase in circulating levels of IL-6 and IL-8. IL-6 levels started to increase at 4th postoperative hour and peaked at 24th postoperative hour. Levels of IL-6 revealed a significant difference regarding IL-6 174G>C genotype. Patients carrying -174 C allele demonstrated a significantly higher plasma concentrations of IL-6 at 4th and 24th postoperative hours. The elevated levels of IL-6 24 hours postoperatively was associated with slightly longer ventilation times although not statistically significant. Similarly Gaudino and colleagues[5] noted increased levels of IL-6 after CPB, renal dysfunction and longer ICU stays in the carriers of -174C allele.

Plasma IL-8 concentrations also started to increase during the time of CPB and remained significantly elevated 24 and 72 hours after the operation. IL-8 can be produced by monocytes, T cells, neutrophils and natural killer cells. It is also produced by endothelial cells, epithelial cells and fibroblasts.[25] Elevated levels of IL-8 during CPB shows an evidence of an inflammatory response however, elevated levels 24 and 72 hours after the operation is an important and interesting finding. This situation may be explained by reperfusion injury. Reperfusion following ischemia of myocardium and lungs leads to the activation of inflammatory cells and these infiltrating and tissue resident cells produce IL-8.[25,26] Another explanation for this elevation of IL-8 levels may be the reexpansion phenomenon that is the reexpansion of the collapsed lungs following CPB.[27]

Although our data revealed an increase in the levels of IL-8 during and after CPB, this increase was not influenced by IL-8 gene polymorphism. There is only little data about IL-8 polymorphism, its influence on IL-8 levels and association with the diseases. Up to our knowledge, most of the studies about IL-8 polymorphism are about its associations with chronic disases as several cancers and pulmonary diseases.[28,29]

It is suggested that IL-8 has an important role in myocardial ischemic injury and more related to CPB than other cytokines.[3] Additionally IL-8 is a potent chemoattractant for leukocytes and may be a cause

of leukocyte sequestration in pulmonary capillary bed.[4] Our data is consistent with this suggestion that perioperative inotrope use, ventilation time, ICU stay and 30-day mortality were significantly related with the elevated concentrations of IL-8.

The present study has some potential limitations, the small number of patients being the most important one. This could be a reason of some of the results that demonstrated no statistical significance. Statistical significance might be increased with larger number of patients. We studied plasma concentrations of IL-6 and IL-8 as markers of SIRS. It would be beneficial to measure other markers of inflammatory response as IL-1β, C5a, C-reactive protein and lactate.

In summary, we report that proinflammatory cytokines, namely IL-6 and IL-8, are associated with systemic inflammatory response syndrome following CPB. Plasma levels of IL-6 is significantly increased in the IL-6 -174C allele carriers. IL-8 polymorphism does not have an influence on the serum levels of IL-8 but elevated levels of IL-8 is strongly associated with poor outcome of the patients following CABG. We can conclude that preoperative determination of of polymorphisms in patients undergoing CABG may lead to various preoperative and postoperative measures for the treatment of systemic inflammatory response. Further studies with larger number of patients are warrented to confirm these findings.

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