The Analysis of Inflammatory Mediator Levels with Modified Hemofiltration in Coronary Artery Bypass Surgery

KORONER ARTER BYPASS CERRAHİSİNDE MODİFİYE HEMOFİLTRA S YON TEKNİĞİ İLE İNFLAMATUVAR MEDİYATÖR DÜZEYLERİNİN DEĞERLENDİRİLMESİ

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Summary

The cardiopulmonary bypass could increase the blood levels of various immune mediators, thereby, could lead a systemic inflammatory response syndrome with some hemodynamic alterations as in sepsis, such as vasodilatation, tachycardia, and decrease in systemic vascular resistance. Perioperative hemofiltration is one of the treatment modalities proposed to prevent this syndrome. It has been recently claimed that the modified hemofiltration is more effective to eliminate the inflammatoiy mediators than the former standard ones. The purpose of this study was to determine the efficacy of modified technique on these mediators.

Forty-two patients undergone coronary artery bypass grafting were equally randomized into two (control and hemofiltration) groups. The blood samples were taken in different control times, as before and after the hemofiltration, to asses the blood concentrations of interleukin-6, interleukin-8 and neopterin.

Although some inflammatory mediators have been filtered and detected in ultrafiltrate analysis of study group, the immune mediator levels did not differ between two groups along the course of study.

The results of our study suggest that whether the modified hemofiltration is more effective in clearence of immune mediators or not is still debatable and questionable.

Key **Words:** Modified hemofiltration, Immune mediators, Coronary disease

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Özet

Kardiyopulmoner bypass çeşitli immün mediyatörlerin kan düzeylerini arttırarak vazodilatasyon, taşikardi ve sistemik damar direncinde azalma gibi sepsiste de görülen bir tür sistemik inflamatuvar yanıt sendromu gelişmesine yol açabilir. Perioperatif hemofiltrasyon, böyle bir yanıtın gelişmesini önleyici bir yöntem olarak gösterilmektedir. Son dönemlerde bu tekniğin modifiye edilmiş şekillerinin inflamatuvar mediyatörlerin temizlenmesinde daha etkili olduğu öne sürülmüştür. Çalışmamızın amacı modifiye tekniğin bu mediyatörler üzerine olan etkinliğini araştırmaktır.

Koroner arter bypass cerrahisi uygulanan 42 hasta eşit ve randomize olarak kontrol ve çalışma (hemofiltrasyon) gruplarına ayrılmışlardır. Hemofiltrasyon öncesi ve sonrası olmak üzere değişik kontrol zamanlarında kan örnekleri alınarak immiin ınediyatörlerden interleukin-6, interleukin-8 ve neopterin düzeyleri araştırılmıştır.

Her ne kadar, çalışma grubunda bir miktar immün mediyatörün filtre olduğu ve ultrafdtrat mayisine geçtiği saptandıysa da, çalışma süresince iki grubun immün mediyatör düzeyleri arasında anlamlı bir fark oluşmamıştır.

Çalışmamızın sonuçları modifiye hemofiltrasyon uygulamasının daha etkili olduğu konusunda bir yargıya varmanın henüz tartışmalı olduğunu ve araştırılması gerektiğini düşündürmektedir.

Anahtar Kelimeler: Modifiychemofıltrasyon, İmmün mediyatörler, Koroner hastalığı

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A systemic inflammatory response syndrome (SIRS) can develop after cardiopulmonary bypass (CPB) operations. This syndrome courses with severely reduced systemic vascular resistance (SVR) demanding vasoconstrictive and fluid replacement

therapy, and increased cardiac output (CO). SIRS is, in fact a delicately complex defense mechanism requiring some factors, such as endotoxins, complement activators, coagulation and fibrinolyzis, neutrophils, elastases and antiproteases, oxygen free radicals, and various cytokins that sometimes cause multiorgan failure (1).

The various preinflammatory cytokins have different effects and metabolisms. Tumor necrosis factor (TNFa) is released from activated monocytes, lymphocytes, and kupffer cells and leads to an increase in oxidative products, degranulation, phagocytosis, and adhesive properties of leukocytes. Interleukin- 6 (IL-6) contributes to regulation of acute phase response, causes fever, and induces ACTH release. Its inliibitory effect on TNFa derived endotoxin could be protective. Interleukin-8 (IL-8) and neopterin are also important cytokins that increase leukocyte adherence and permeability, thereby, lead some postoperative organ dysfunctions (2).

As a protective method against CPB derived inflammatory response and eventually developed myocardial and pulmonary edema, the hemofiltration was introduced in 1970's, following the first successful study of Magilligan (3).

Standard hemofiltration is a process separating the water and particles in low molecular weight from blood through a semipermeable membrane, according to hydrostatic pressure gradients. This standard one is performed by replacement of inlet of ultrafilter distally to oxygenator and outlet directed to venous reservoir. Ultrafiltration starts subsequent to beginning of rewarming. Nevertheless, it was claimed that this conventional technique had failed to reach desired benefits; thus, some new modifications come into current practice. One of them as well as performed by us, is to establish the hemofiltration after the completion of CPB, during 10-15 min. intervals via settlement of the inflow on arterial line close to aortic cannula and of outflow to directly right atrium. Therefore, hemofiltcr lines become shorter and ultrafiltration run with own systemic pressure of patient without any need of extra pump head. It is asserted that the desired increase in hematocrit levels with corresponding reduce in transfusion needs are more efficiently provided with this technique (4). However, there are still arguable results among studies inquiring which technique is most effective in both removing inflammatory mediators and improving hemodynamic performances.

It is well known theoretically that particles only having less than 20 kD molecular weight can be ultrafiltrable and molecular weights of IL-6, TNFa and IL-8 are 20-30, 17-50 and 8-10 kD respectively. Therefore ultrafiltration of various particles could not be explained with only molecular weights. Probably complex formations of molecules are also effective in diffusion gradients (5).

Although it was claimed in many journals that hemofiltration was enable to remove endotoxins, cytokins, and some other myocardial depressant factors in not only CPB performed patients, but also in septic and multiorgan failure cases, a significant difference in plasma cytokin levels could not be detected between hemofiltration performed and unperformed cases. Despite unclarificd effect mechanism, the hemodynamic performances of hemofiltration performed patients are superior than those of unperformed ones (6, 7).

There are many fiber materials (i.e. polyamide, polyacrilonitrile, polysulfon) as well as fiber types (i.e. hollow fiber, multilayer flat plate) in current practice. These fiber types have different effects on clearance of different cytokins. Inflammatory mediators have different behaviours in hemofiltration. Ultrafiltration having similiar functional capacity in chronic renal failure, run in different capacities in septic patients. It is wise to say that the effective clearances of different mediators require different and appropriate filters. Moreover, changing mediator profiles during progression of a disease require to use different filter types adjusted to profile (7).

This study was designed in a controlled fashion with the aim of assessing the effects of modified hemofiltration on removal of IL-6, IL-8 and neopterin, which are inflammatory cytokins, from blood of coronary artery bypass grafting (CABG) performed patients.

Material and Methods

We studied 42 patients undergone elective CABG. The patients were prospectively randomized and allocated equally into study (ultrafiltration)

and control groups. The work was approved by ethic committee of this hospital and informed consents were obtained from all patients. The patients who underwent emergency surgery, having diagnosed systemic disorder such as, hemostatic defect, hypertension, diabetes and renal failure were excluded. There was not any significant difference between two groups regarding age, gender, ejection fraction (EF), cross clamp, CPB times and initial hematocrit levels.(Table 1).

Anesthetic induction was performed with 5 mg/kg fentanyl, 3-5 mg/kg thiopental sodium, 1 mg/kg lidocain and 0.1 mg/kg pancuronium and enflurane was added as inhalatory anesthetic in management of anesthesia. Not once was any inotropic support used, and only Na-nitroprussid was used as antihypertensive management, if required, during CPB and immediate postoperative period. A11 operations were performed through median sternotomy incision. The membrane oxygenator was primed with 2 L of lactated Ringer's solution. CPB was established via standard aortic and single venous cannulation using a Sams modified roller pump (Sarns, Ann Arbor, MI, USA). During CPB, oxygenation was achieved with a D 708 Simplex adult hollow-fibre oxygenator (Dideco, Mirandola, Italy), and a 40 mm blood filter (Dideco, Mirandola, Italy) was used on the arterial line. During bypass, the hematocrit was maintained between 20% and 25%, non-pulsatile pump flow between 2.0 and 2.5 L/min/m2, and mean arterial pressures between 50 and 65 mmHg. After the aortic cross-clamping, all patients received intermittant, moderately hypothermic blood cardioplegia. Topical hypothermia was also used in all operations. Body temperature was maintained between 28 and 30°C during CPB. Distal anastomoses were performed during a complete period of aortic cross-clamping and proximal anastomoses were performed with partial aortic clamping during rewarming. Only internal mammarian artery and greater saphenous vein grafts were used in all cases. Any cell-saver application was not needed along the course of study.

Modified hemofiltration was performed via ultrafiIters, filtering particles only having 15 to 50 kD in molecular weight and membrane materials of made from polyacrilonitrile (AN 69). In study group hemofiltration was commenced after CPB, but before the heparin neutralization, at rewarming period and continued during 10 to 15 minutes. The arterial blood was delivered to hemofilter through a line connected directly to aortic cannula with running force of only patients own systemic pressure and filtered blood was drained directly to right atrium. The systemic pressure of patient obviated the need for an extra pump head.

In study group, blood specimens were taken in scheduled times as, after the CPB, during hemofiltration, postoperative 3rd hour, and postoperative 20th hour; in control group, however, these were taken as preoperatively, before the protamine neutralization, postoperative 3rd hour, and in postoperative 20th hour via sampling from radial artery and were processed with ELISA system in Sorin Biomedica device. The evaluations for IL-6 were made with preservation of specimens at -70 °C and computed as pg/ml by means of Biotrak IL-6 human elisa system kits (Amersham International pic, Buckinghamshire, England). The specimens for LL-8 evaluations were preserved under -20 °C and computations were made as pg/ml by means of Quantikine Human IL-8 Immunoassay kit (R&D Systems, Inc. Minneapolis, MN, U.S.A.). The neopterin measurements were made as nmol/ml via evaluation of specimens preserved at 2 to 8 °C by means of IBL Neopterin Elisa kit (IBL Gesselschaft fur Immunchemie und Immunbiologie mbH, Hamburg, Germany).

The postoperative volume replacement was performed with either blood, if hematocrit level was below 28%, or fresh frozen plasma, if there was a drainage exceeding 2 mg/kg/h and hematocrit level was above 28%.

Statistical analyses were performed via SPSS (ver. 5.0.1) program. The probability less than 0.05 was considered significant. The mean and standart error of mean (SEM) values of all parameters were calculated and indicated. The Friedman Two-Way Annova Test was used to evaluate the change in immune mediator levels proportional to sampling times. The Maim-Whitney U-Wilcoxon Rank Sum W Test was applied to evaluate the differences of clinical fixed parameters (age, CPB, EF, X clamp time). The Fisher's exact test was performed to compare the two groups in nominal calculations and in detected relative risk rates.

Results

There was not any significant difference between two groups regarding basic demographic and operative characteristics (age, gender, CPB, X clamp, EF) (Table 1). There was not any mortality and morbidity in both groups also.

The effects of modified hemofiltration

There was not any significant difference in comparison of blood levels of IL-6, IL-8, and neopterin in different sampling times as either between two groups or in each group (Fig 1A, IB, 1C).

Although the modified ultrafiltration process had not caused a significant change in inflammatory mediator levels between two groups, we observed that some inflammatory mediators could have been filtered and thereby eliminated. In the analysis of ultrafiltrate, the filtered amounts were 1.32±0.63 pg/ml for IL-6, 7.28±3.59 pg/ml for IL-8, and 0.14±0.08 nmol/ml for neopterin.

It was considerable among postoperative observation data that the mean hematocrit (htc) levels of hcmofiltercd group soon after hcmofiltration (32.9±0.2% versus 28.8±0.3%; p=0.0082) and at postoperative 3rd hour (33±0.4% versus 29.3±0.2%; p=0.00T) were significantly higher than those of control group. In comparison of the postoperative blood transfusion need, it was detected as 0.84±0.31 and 1.85±0.29 units for hemofiltercd and control group, respectively. This difference was also significant as in comparison of htc levels (p<0.05). There was not any significant difference, however, regarding other data such as postoperative total urination, drainage, cardiac arrhytmia and inotropic requirements.

In the study group, the mean hemofiltered volume was 1110± 28.17 ml and the mean hemofiltration time was 23.34±1.22 min. being compatible with literature.

Table I. Operative and demografic data of the patients

	Study n=21	Control n=21	p value
Age	58,3± 3,85	61,27± 3,51	NS
Sex (male/female)	16/5=%(77/23)	15/6=%(72/28)	NS
EF (%)	43,14 ±3,41	43,79± 3,69	NS
Cross-clamp time (min)	53,10± 5,11	49,28 ±6,19	NS
CPB time (min)	74,15± 8,31	71,43 ±6,91	NS
Hematocrit (%)	43.21±3.92	43.55 ±4.11	NS

EF: ejection fraction, CPB: cardiopulmonary bypass.

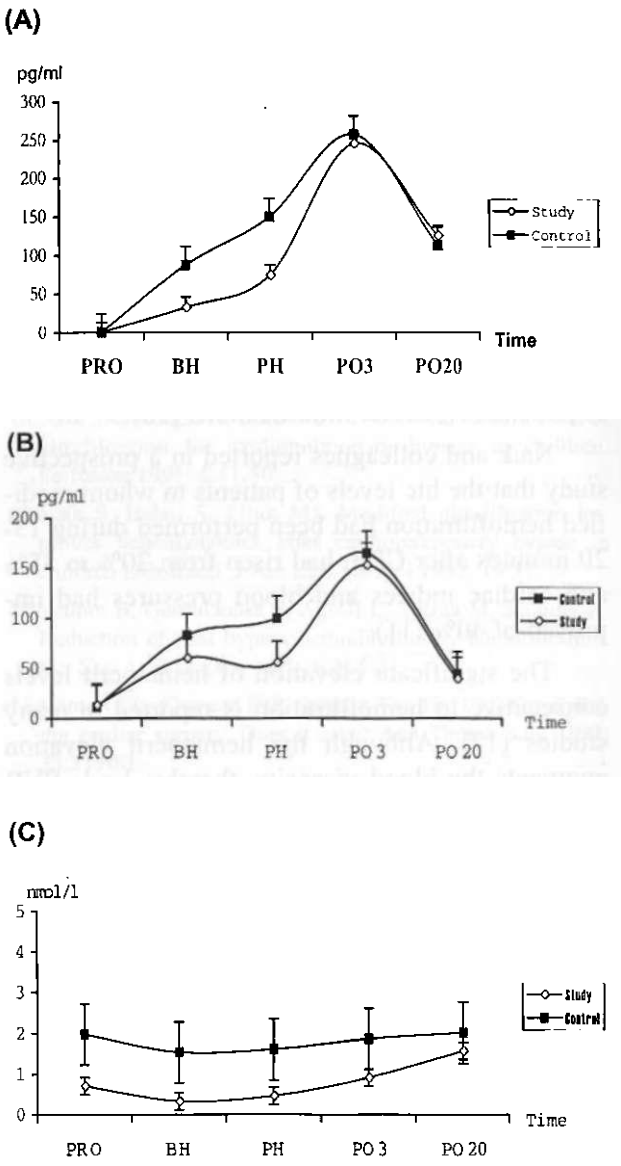

Figure 1. Alterations of the plasma concentrations of interleukin-6 (IL-6) (A), interleukin-8 (IL-8) (B) and neopterin. (PRO: Preoperative, BH: Before hemofiltration, PH: Post-hemofiltration, P03: Postoperative 3rd hour, PO20: Postoperative 20th hour.)

Discussion

There is some evidence that cytokine levels may relate to myocardial ischaemia after cardiac surgery (8). The inflammatory theory after myocardial ischacmia/reperfusion is, however, supported by annual studies (9). When, the standart hemofiltration failed to reach desired benefits as a preventive measure against such an inflammatory response, modified techniques come into current practice and it was asserted in earlier studies that the desired htc levels could be reached at least 40% of patients, by newer modifications (10).

Naik and colleagues reported in a prospective study that the htc levels of patients to whom modified hemofiltration had been performed during 15-20 minutes after CPB, had risen from 20% to 35% and cardiac indices and blood pressures had improved of 40% (11).

The significant elevation of hematocrit levels consecutive to hemofiltration is reported in many studies (12). Although the hematocrit elevation augments the blood viscosity, thereby, leads SVR elevation and negatively effects myocardial functions, it can function as a protector by enhancing the oxygen supply to tissues and myocardium (4). The improvement in cardiac performances might be explained with concomittant decrease in myocardial edema coupled with elevation in hematocrit levels rendering qualified oxygen transfer. We also observed that the hematocrit levels of hemofiltcred group were significantly higher as well as the blood transfusions were significantly less than the control group during all postoperative period. In this respect, ultrafiltration is more cost effective than the cell saver (13).

The eliminativc effects of hemofiltration on various cytokins appearing as a response to CPB and leading a SIRS like sepsis, were suggested in many studies. However, there are discordant results regarding the efficacy spectrum of ultrafiltration on various cytokins, among published reports (4, 5).

The detection of cytokins after CPB procedures, that normally invisible in blood was explained with either ongoing endotoxemia secondary to bowel ischaemia or complement activation secondary to contacting of blood with foreign surfaces. Moreover, it was proved that the release of cytokins increased proportionally with CPB time (14). It is also asserted that the measurement of various cytokins, such as neopterin, is useful to monitor disease development in patients with myocardial inflammation (15).

Millar and colleagues suggested that the IL-6 and TNFa could be eliminated by ultrafiltration, * whereas others reported IL-8 could not be changed, even some others asserted the hemofiltration could cause the elevation of the cytokins (16). The cause of different results in such a wide spectrum from cytokin elevations to reductions, is still debated. It must be remembered that the mean half life of cytokins as short as 15 minutes (17). Probably, the spontaneously inactivated ones are more abundant than the hemofiltcred fraction, therefore hemofiltered amount is negatively affected. Besides, there arc some other cytokin components in plasma, undetectable by ELISA, such as ones in polymeric phase with aggregates. The more elimination of free fraction causes the more releasing of polymeric fraction, thereby, plasma cytokin level can rise up unproportionally with hemofiltered amount (18).

Barrera and colleagues reported that the elimination of IL-ip and TNFa was most prominent at first several minutes of filtration, thereafter the membrane was saturated completely with cytokins (21). The rapid saturation of cytokin bounding process can explain the gradual decrease in effectiveness of hemofiltration. Moreover, the filter type also affects the measured cytokin levels, for example, the elimination of IL-6 by polyacrilonytril filter is more effective than that by polyamide filter. Consequently, different mediators need different filter types for clearance. The immune mediators take part in different stages of immune process, for example, the maximum level of IL-8 in plasma is detected at postoperative 4th hour that match the time subsequent to activation of IL-8 by TNFa. Consequently, it is possible to suggest that a filter effectively eliminating the TNFa could be ineffective to eliminate IL-8, thus, in later periods of hemofiltration, another filter will be needed to eliminate IL-8 (7, 16).

Theoretically, the particles only having less than 20 kD in molecular weight can be ultrafiltrablc. Since the molecular weights of most of the immune mediators are close to these levels, it is al-

so asserted that the ultrafiltration process depends on not only molecular weights but also the shapes and filtration constants of molecules (20).

The capability of modified hemofiltration to prevent the occurrence of a systemic inflammatory reaction after CPB, by reducing inflammatory mediator levels, thereby saving of SVR and PVR levels and avoiding of oncoming hyperdynamic heart failure, is suggested in many reports (1, 2, 4, 16). Nevertheless, there are still some other reports suggesting that the elimination of excessive fluid load is only effect mechanism of hemofiltration. Moreover, whether the modified techniques are superior than the conventional ones or not, is still vague (5).

We did not observe a significant difference between study and control groups in all samples regarding IL-6, IL-8 and ncopterin levels, although small amounts of them were detected in filtrate. The asserted improvement in cardiac performances, in former studies, might be related to both prevention of hypervolemia and qualified oxygen transfer provided by hematocrit elevation.

Our data suggest that the effects of modified hemofiltration on blood levels of inflammatory mediators in CPB procedures are still debatable and the exact cardioprotective mechanism needs to be clarified.

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