Effects of Extracorporeal Circulation on Thrombin-Antithrombin Ⅲ and Prothrombin Fragment 1+2 Levels

EKSTRAKORPOREAL DOLAŞIMIN TROMBİN-ANTİTROMBİN III KOMPLEKSİ VE . PROTROMBİN 1+2 DÜZEYLERİ ÜZERİNE ETKİSİ

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Summary

Cardiopulmonary bypass may alter the factors responsible for normal haemostasis by exposing blood to non-endothelial surfaces, eg. extracorporeal circuits. In order to evaluate the probable effect of extracorporeal circulation on haemostasis, we measured thrombin-antithrombin III complex (TAT) and prothrombin fragment 1+2 (PF 1.2) plasma levels which are the biological markers of in vivo coagulation, in 16 patients who underwent coronary artery bypass grafting (CABG). Postoperative PF 1.2 were higher in comparison to the pre-operative concentrations of the parameter. Pre- and post-operative TAT concentrations showed no statistically significant difference. The increment in the PF 1.2 levels in CABG patients might suggest ongoing subclinical haemostatic activation associated with CABG. Further investigations are needed to clarify the exact relationship between increased PF 1.2 levels and thrombotic complications observed in CABG patients. Haemostasis in CABG is still an enigma and remains to be elucidated.

Key **Words:** Coronary artery bypass grafting, Extracorporeal circulation, Thrombin-antithrombin III complex, Prothrombin fragment 1+2, Thrombotic tendency

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Özet

Kardiyopulmoner bypass sırasında olduğu gibi kanın endotel dışı yüzeylerle teması, normal hemostazdan sorumlu faktörlerde değişikliklere yol açabilir. Bu çalışmada ekstrakorporeal sirkülasyonun hemostaz üzerine bu olası etkisini saptamak için, in vivo koagulasyonun biyolojik belirleyicileri olan trombin-antitrombin III kompleksi (TAT) ve protombin fragman 1+2'nin (PF 1.2) plazma düzeylerini koroner arter bypas greftlemesi (CABG) yapılan 16 hastada ameliyat öncesi ve sonrası dönemde belirledik. PF 1.2'nin serum düzeyipostoperatif dönemde preoperatif döneme göre anlamlı bir şekilde artarken, TAT kompleksindeki postoperatif artış, istatistiksel olarak anlamlı değildi. PF 1.2 seviyelerindeki bu artış CABG süreci ile ilişkili subklinik bir hemostatik aktivasyonun göstergesi olabilir. Ancak açık kalp cerrahisi geçiren hastalarda görülen trombotik komplikasyonlarla PF 1.2 seviyelerindeki bu değişiklikler arasındaki ilişkileri açıklamak için daha ileri çalışmalara ihtiyaç vardır. CABG ve hemostaz ilişkisi halen tümüyle aydınlatılamamış karmaşık bir konudur.

Anahtar Kelimeler: Koroner arter bypass cerrahisi, Ekstrakorporeal dolaşım, Trombin-antitrombin III kompleksi, Protrombin fragman 1+2, Tromboza eğilim

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Cardiopulmonary bypass replaces the functions of the heart and the lung in open heart surgery and the circuitry consists of a pump, a disposable oxygenator, reservoirs, and tubing systems. The normal functions of blood flow, gas exchange, blood surface interface effects and reticuloendothelial function are replaced totally or in part by the extracorporeal bypass. Nonphysiological parameters of the extracorporeal circulation (ECC) are the nonpulsatile flow, anticoagulation, haemodilution, hypothermia and relative hypoperfusion. During ECC the blood is exposed to large foreign surfaces and materials and flow conditions normally not found in the circulation (1). All of these factors af-

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feet the platelet function, the coagulation and fibrinolytic system. Normally, there is a continuous fibrin formation on the endothelial surface which is balanced with concomittant fibrinolysis. If it is upset clotting mechanisms takes place. Fibrinogen is absorbed to the circuit surfaces which later on binds platelets and clotting mechanism is activated and plasminogen activation and fibrinolysis occurs (1,2). Heparinization is used to prevent the blood coagulation during ECC. Initially the levels of factors V, VIII, antithrombin III, plasminogen, alpha-2 antiplasmin decrease during ECC which is due to hemodilution, subsequently increase due to synthesis by the liver. Platelet concentration drops during the ECC. Platelet functions alter (insensitive to ADP and epinephrine) and activation occurs (1,3). Depending upon above-mentioned interrelationships between haemostasis and open heart surgery, we aimed to investigate haemostasis in open heart surgery. To assess whether there is a subclinical activation of the haemostatic system associated with open heart surgery, in vivo molecular markers of haemostatic activation (namely, prothrombin fragment 1+2 and thrombin-antithrombin III complex) were determined preoperatively and at the end of the cardiopulmonary bypass in operated patients (4).

Patients and Methods

The study was carried out on sixteen patients [14 men and 2 women (ages from 38 to 67 years)] who underwent CABG in Hacettepe University Thoracic and Cardiovascular Surgery Department. The patients with impaired renal function, with bleeding diathesis, who receiving anticoagulant therapy (salicylic acid, heparin, warfarine, nonsteroid antiinflammatory drugs) and fibrinolytic medication and whose cardiopulmonary bypass time less than 60 minutes were not included into the study. In all patients, Univox membrane oxygenator (Bentley, Irvine CA, USA), De Bakey roller pump and Bentley' Duraflo II pump systems were used. The patients were cooled to 28° C and cold potassium cardioplegia was applied. The perfusion pressure was kept above 40 mm Hg. Bypass flow rates were maintained at 2.4 L/m2/min. Heparinization 3 mg/kg (Liquemine®, Roche) was applied before cannulation and ACT was kept above 400 seconds (Hemachrone, International Technidyne Corp. Edison, NJ, USA) and neutralization of heparin after the bypass was achieved by protamine hydrochlorure (Protamin®, Roche) administration. Blood specimens were obtained from the patients before the bypass procedure and 24 hours after the operation and plasma prothrombin fragment 1+2 (PF 1.2) and thrombin-antithrombin 1ll complex (TAT) levels were determined. PF 1.2 and TAT concentrations in plasma samples were measured by solid phase sandwich ELISA method (Enzygnost F 1+2 and Enzygnost TAT microenzyme immunoaasay, Behringwerke AG, Germany).

Results were expressed as mean ± standard deviation (SD). Statistical significance was assigned to p concentrations lower than 0.05. For comparison of the pre- and post- operative PF 1.2 and TAT concentrations, Wilcoxon test was used. SPSS (Statistical Package for Social Sciences) for Windows v5.0 was used to analyse the data.

Results

The pre- and post-operative concentrations of TAT and PF 1.2 in plasma were compared. Post-operative plasma concentrations of PF 1.2 were higher in comparison to pre-operative concentrations of the parameters. Pre- and post-operative TAT concentrations showed no difference statistically. Results were shown in Table 1.

Discussion

The exposure of blood to foreign surfaces during cardiopulmonary bypass leads to activation of coagulation and fibrinolytic systems. Despite a normal clotting mechanism before operation many pa-

Table 1. Haemostatic parameters in the pre- and post-operative state of coronary artery bypass graft surgery

	Preoperative	Postoperative	p
Thrombin-antithrombin III complex (TAT), mgr/dL	4.37+2.44	4.46+3.52	0.08
Prothrombin fragment 1+2 (PF 1.2), nmol/L	2.87+1.02	3.82+2.59	0.01

tients suffer from post-operative bleeding while laboratory measurements of haemostasis show only mild abnormalities and no specific deficiencies. PF 1.2, the peptide fragment generated when prothrombin is activated to thrombin reflects thrombin generation and is found during the activation of coagulation and indicates some fibrine has already formed. After thrombin formation it forms complexes with a serine protease inhibitor, antithrombin 111, forming TAT. These two markers of coagulation circulate in the blood of patients who suffer from any kind of thrombotic disorders. In vivo molecular haemostatic markers show hemostatic system activation. In our study, the increment in the PF 1.2 levels in CABG patients was statistically significant. This finding might suggest ongoing subclinical haemostatic activation associated with extracorporeal circulation. On the other hand, the increment in TAT concentration after 24 hours of CABG was not statistically significant. Our results are in concordance with the results of the other studies (5-7). Some studies report that the changes in the levels of these hemostatic markers continue for up to 5 days postoperatively (6). Further investigations are needed to clarify the exact relationship between increased PF 1.2 levels and thrombotic complications observed in patient who underwent CABG surgery. Haemostasis in open heart surgery is still an enigma remains to be elucidated. The data suggests that open heart surgery induced hypercoagulability due to excessive thrombin generation.

REFERENCES

1. Kay P H. Techniques in extracorporeal circulation. Third edition. Butterworth- Heinemann Ltd., 1992:144-55. ,
2. Paramo JA, Rifon J, Llorens R, et al. Intraopereative and postoperative fibrinolysis in patients undergoing cardiopulmonary bypass surgery. Haemostasis 1991; 21: 58-64.
3. Edmunds L H , Ellison N, Colman R W, et al. Platelet function during cardiac operation, Comparison of membrane and buble oxygenators. J Thorac Cardiovacs Surg 1982; 83: 805-12.
4. Fareed J, Brick R L, Hoppensteadt D A, et al. Molecular markers of hemostatic activation; applications in the diagnosis of thrombosis and vascular and thrombotic disorders. Clin Appl Thromb Hemostas 1995; 1: 87-102.
5. Slaughter TF, Le Bleu TH, Douglas JM, et al. Charecterization of prothrombin activation during cardiac surgery by hemostatic molecular markers. Anesthesiology 1994; 80: 520-6.
6. Rifon J, Paramo JA, Prosper I, et al. Thrombin-antithrombin complexes and prothrombin fragment 1+2 in aortocoronary bypass surgery relation to graft occlusion. Hematol Pathol 1994; 8: 35-42.
7. Welters I, Menges T, Ballesteros M, Knothe C, Ruwoltt R, Gorlach G, Hempelmann G. Thrombin generation and activation of the thrombomodulin protein C system in open heart surgery depend on the underlying cardiac disease. Thromb Res 1998;92:1-9.