RESEARCH ARTICLE / *Araştırma Makalesi*

Relationship Between the Coronary Artery Bypass Graft Operation and the Levels of Ceruloplasmin Enzymatic Activity as an Antioxidant

Koroner Arter Baypas Greft Operasyonu ile Antioksidan Olarak Seruloplazminin Enzimatik Aktivitesi Arasındaki İlişki

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Abstract

Objective	Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism. Moreover, its antioxidant activity was showed in several studies. In this study, we investigated the relationship between coronary artery bypass graft operation and the levels of ceruloplasmin enzymatic activity.
Materials and Methods	The study included 120 patients who underwent coronary artery bypass graft operation. Peripheral blood samples were taken preoperatively, peroperatively –before and after cardiopulmonary bypass-, at postoperative 4th hour, 3rd day and 5th day. Blood samples before and after cardiopulmonary bypass were also taken from the coronary sinus. The enzymatic activity of ceruloplasmin levels were measured by Erel method.
Results	There was a gradual decrease in the enzymatic activity of ceruloplasmin levels after cardiopulmonary bypass. In the postoperative 4th hour and the 1st day, reduction was statistically significant in the blood samples taken peripherally (p=0.025). Activities increased in the postoperative 3rd and 5th days, respectively. According to the coronary sinus blood samples, there was also a significant decrease in the enzymatic activity of ceruloplasmin after cardiopulmonary bypass (p=0,035).
Conclusion	Our study showed significant differences in preoperative, peroperative and postoperative levels of the enzymatic activity of ceruloplasmin. The reduction in postoperative period during the 3rd day which was probably due to consumption as a respond to oxidant activity, demonstrated that cardiovascular bypass significantly increased oxidative stress. Enzymatic activity of the ceruloplasmin may be one of the parameters that reflect the response of inflammatory and oxidative stress associated with cardiopulmonary bypass.
Keywords	ceruloplasmin; cardiopulmonary bypass; coronary artery bypass graft operation; enzymatic activity; oxidative stress

Öz

Amaç	*Seruloplazmin, kanda bakır taşıyan başlıca proteindir ve demir metabolizmasında da rol oynar. Ayrıca antioksidan aktivitesi birçok çalışmada gösterilmiştir. Bu çalışmada koroner arter baypas greft operasyonu ile seruloplazminin enzimatik aktivite düzeyleri arasındaki ilişkiyi araştırdık.*
Gereç ve Yöntemler	*Çalışmaya koroner arter baypas greft operasyonu geçiren 120 hasta dahil edildi. Periferik kan örnekleri ameliyat öncesi, ameliyat sonrası - kardiyopulmoner baypas öncesi ve sonrası-, ameliyat sonrası 4. saat, 3. gün ve 5. gün alındı. Koroner sinüsten de kardiyopulmoner baypas öncesi ve sonrası kan örnekleri alındı. Seruloplazmin düzeylerinin enzimatik aktivitesi Erel yöntemi ile ölçüldü.*
Bulgular	*Kardiyopulmoner baypas sonrası seruloplazmin düzeylerinin enzimatik aktivitesinde kademeli bir azalma oldu. Postoperatif 4. saat ve 1. gün periferden alınan kan örneklerinde azalma istatistiksel olarak anlamlıydı (p = 0,025). Aktiviteler sırasıyla postoperatif 3. ve 5. günlerde arttı. Koroner sinüs kan örneklerine göre de, kardiyopulmoner baypas sonrası seruloplazmin enzimatik aktivitesinde önemli bir azalma vardı (p = 0.035).*
Sonuç	*Çalışmamız, seruloplazmin enzimatik aktivitesinin preoperatif, peroperatif ve postoperatif düzeylerinde önemli farklılıklar gösterdi. Muhtemelen oksidan aktiviteye yanıt olarak tüketime bağlı olan postoperatif 3. gündeki azalma, kardiyovasküler baypasın oksidatif stresi önemli ölçüde artırdığını göstermiştir. Seruloplazmin enzimatik aktivitesi, kardiyopulmoner baypas ile ilişkili enflamatuar ve oksidatif stresin tepkisini yansıtan parametrelerden biri olabilir.*
Anahtar Kelimeler	*seruloplazmin; kardiyopulmoner baypas; koroner arter baypas greft operasyonu; enzimatik aktivite; oksidatif stres*

INTRODUCTION

Morbidity and mortality are highly related with ischemia/reperfusion injury, surgical trauma and patient characteristics in cardiac surgery.[1] Oxidative stress is caused by an imbalance between antioxidants and free radicals and commonly seen in major surgical operations and especially in coronary artery bypass graft operation (CABG) and alters normal endothelial functions. It induces proinflammatory, proliferative, prothrombotic, and vasoconstrictor mechanisms that form atherogenic processes. Beside these, oxidative stress and inflammatory response caused by cardiopulmonary bypass may result in myocardial damage and variable grade myocardial dysfunction.[2,3] In the literature, insufficient antioxidant defense and oxidative stress are reported in the pathogenesis of cardiovascular diseases.[4] Advanced age, significant co-morbidities and the cardiopulmonary bypass procedures are also associated with postoperative complications, increased length of hospital stay and enhanced degree of oxidative stress.[5]

The formation of Reactive Oxygen Species (ROS) after ischemic reperfusion triggers a chain of complex events. These oxidative events lead to increased lipid peroxidation, decrease of plasma antioxidants and the formation of other harmful metabolites.[1]

Ceruloplasmin (Cp) is an acute phase reactant with alpha-2-glycoprotein structure with a molecular weight of about 132 kDa.[6] It has ferro-O^2-oxidoreductase activity directed towards ferrous ion stimulated lipid peroxidation and formation of hydroxyl radical in Fenton reaction.[7] Cp has diverse functions. The known functions of Cp include copper transportation, iron metabolism, anti-oxidation, angiogenesis and coagulation. Cp is also effective in regulating vascular tone.[6] Since Cp inhibits nitric oxide (NO) synthesis, it can modify the vascular responses through NO.[8] Cp can also act as a pro-oxidant or an antioxidant. It catalyzes the oxidation of Fe^{+2} to Fe^{+3}.[9] It has also an antioxidant effect on LDL by blocking Cu^{+2}-induced lipid oxidation.[2]

The current study was designed to establish the relationship between cardiopulmonary bypass operation and the enzymatic activity of Cp levels in preoperative, preoperative and postoperative period in patients underwent CABG.

MATERIALS and METHODS

Patient Selection

The study was conducted prospectively between January 2016 to June 2018. The study was approved by the ethics committee of clinical research of Harran University with the decision no: 02-12-2010-06. The study population consisted of 120 consecutive patients undergoing coronary artery bypass graft surgery. Written informed consent was obtained from all participants. Patients' demographics were recorded. Patients with low ejection fraction (<25%), neoplastic disease, systemic inflammatory disease, infection, chronic obstructive pulmonary disease, major depression, liver and kidney disease, cardiac valve procedure, congenitally cardiac procedure, emergency cardiac procedure, off-pump CABG and recurrent cardiac surgery were excluded from the study.

Anesthetic and Surgical Techniques

Midazolam (10 mg/kg) 3 hours before the operation was administered intravenously to the patients for premedication. No other premedication was applied. Anesthesia consisted of a balanced opiate based general anesthesia technique. Propofol (1.5-2.2 mg/kg), rocuronium (0.6 mg/kg), and fentanyl (3 mg/kg) infusions were administered in induction. During the operation, anesthesia was maintained with continuous propofol infusion (10-20 ml/h), remifentanil (0.25-1 mg/kg) and rocuronium. Hemodynamic parameters were kept constant. Median sternotomy was performed under general anesthesia. The cannulation was done with aortic cannula and two stage venous cannula. Standard cardiopulmonary bypass was applied with mild hypothermia (26-32°C) and isothermic blood cardioplegia. Cardioplegia was performed by antegrade way from the aorta and retrograde way from the coronary si-

nus. Cardioplegia application was repeated in every 15-20 minutes. All the cases were applied with perfusion pressure was kept control of at 60-70 mm Hg. Full cardiac flow was maintained with 2,4 L/m^2. Routine heparinization was performed in operation (300 IU/kg). Throughout the operation, activated clotting time (ACT) was kept over 480 seconds. Standard protamine sulfate was applied at the exit from the cardiopulmonary bypass (120-150 IU). Intensive care follow-up of all patients were performed as standard. Cross clamp time, total operation time, cardiopulmonary bypass time, intensive care and hospitalization duration were recorded.

Blood Sample Collection and Enzymatic Activity of Ceruloplasmin Measurements

Blood samples were taken peripherally from all patients 24 hours before the operation, intraoperative-before cardiopulmonary bypass, after cardiopulmonary bypass-, postoperative 4th hour, postoperative 24th hour, postoperative 3rd day and postoperative 5th day for biochemical analysis. Blood samples before and after cardiopulmonary bypass was also taken from coronary sinus. Samples were kept at room temperature for 30 minutes and then separated from the cells by centrifugation at 3000 rpm for 5 minutes. Serum samples were stored at -80°C until the day of biochemical analysis. The enzymatic activity of Cp was measured by Erel method.[10,11] In this method, the iron ion is oxidized to ferric ion by Cp ferroxidase activity. Results were recorded as U/ml.

Statistical Analysis

SPSS 11.5 software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Student's t test was used in the statistical analysis of coronary sinus blood samples. Repeated measurement of variance was performed of Cp level of venous blood samples. Arithmetic averages of all parameters were given with standard deviation values. P values less than 0.05 were considered significant.

RESULTS

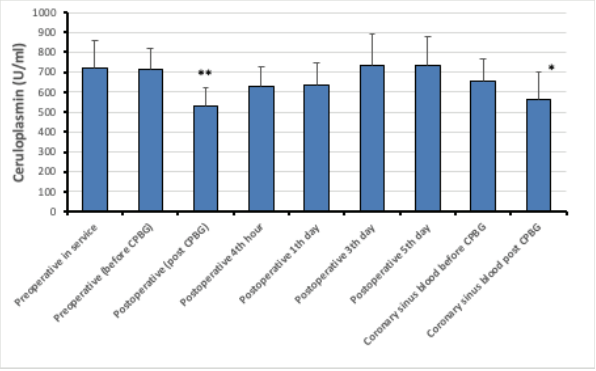
Mean cross clamp time was 88.13 ± 31.70 (min-max 46-175) minutes, mean cardiopulmonary bypass time was 133.83 ± 45.67 (min-max; 70-210) minutes, mean hospital stay was 10.53 ± 5.28 (min-max; 1-27) days, mean duration in intensive care unit was 2.33 ± 1.56 (min-max 0-9) days. The mean ejection fraction was 47.63 ± 11.35% (min-max 25-73). The mean number of anastomosis was 2.73 ± 0.69 (min-max; 2-4). Demographic data, duration of operation, postoperative intensive care and hospital stays are summarized in Table 1.

Table 1: Demographic data, duration of operation, postoperative intensive care and hospital stays of the patients

Variables	Patients (n=120)	S.D. or %
Age(years)	61.80 (41-77)	±9.88
Gender (M/F)	67/53	%56.25
Diabetes (yes/no)	37/83	%31.2
Hypertension (yes/no)	63/57	%53.2
Smoking status (yes/no)	60/60	%50
Dyslipidemia (yes/no)	57/63	%46.8
Mean X-clemp time (min)	88.13(46-175)	±31.70
Mean CPBP time(min)	133.83(70-210)	±45.67
Hospital stay (length)(day)	10.53(1-27)	±5.28
ICU stay (length)(day)	2.33(0.00-9.00)	±1.56
Ejection fraction (%)	47.63(25-73)	±11.35
Previous MI (yes/no)	60/60	%50
Number of anastomosis	2.73(2-4)	± 0.69

CPBP: Cardiopulmonary bypass pump
ICU: Intensive care unit
CABG: Coronary artery bypass graft
MI: myocardial infarction

In our study, there was a decrease in enzymatic activity of ceruloplasmin levels immediately after cardiopulmonary bypass both in the samples taken peripherally and from coronary sinus. This decrease continued until 24 hours after the operation ($p < 0.05$). Postoperative 3rd day, the enzymatic activity of the ceruloplasmin becomes the normal values. Reduction in blood samples from the coronary sinus was observed to be more apparent ($p < 0.05$). Enzymatic activity of Cp levels are presented in Figure 1.



**p=0,035

* p=0,025

Figure 1:Enzymatic activity of ceruloplasmin levels

DISCUSSION

Ceruloplasmin (Cp) is an acute phase reactant and it acts as a pro-oxidant or an antioxidant. Many investigators have reported that coronary artery bypass graft operation leads to increase in oxidative stress.[1] Normotensive-normotermic condition followed by hypotensive-hypothermic period leads to ischemia-reperfusion injury and this mechanism causes oxidative stress especially in on-pump CABG.[12] Mumby and colleagues studied the enzymatic activity of Cp levels in 65 patients with congenital heart disease. They investigated the enzymatic activity of Cp before, during and after cardiopulmonary bypass. It was showed that the enzymatic activity of Cp levels decreased during cardiopulmonary bypass and increased postoperatively.[13] Jeremy et al.[14] studied the enzymatic activity of Cp levels in 55 adult patients with coronary artery disease. They evaluated the enzymatic activity of Cp levels at preoperative, postoperative 1st day, postoperative 6th day and postoperative 6th week. They detected a decrease in levels on the postoperative 1st day. On postoperative 6th day and at postoperative 6th week, they found that the enzymatic activity Cp values are higher than preoperative Cp values.[14] Lull et al. studied on the enzymatic activity of Cp values in 10 pediatric patients with congenital heart disease. They reported that levels were reduced by 40% during cardiopulmonary bypass but after 24 hours, it reached normal levels.[15] According to the study conducted by Melnikov et al. in patients with congenital heart disease, the levels of Cp enzymatic activity decreased during cardiopulmonary bypass and returned to normal after 24 hours after cardiopulmonary bypass.[16] Hepponstall et al. reported that in children with tetralogy of Fallot, levels of the enzymatic activity of Cp decreased at the 6th and 12th hours after cardiopulmonary bypass.[17]

Our study demonstrates preoperative and postoperative marked changes in the enzymatic activity of Cp levels. Reduction continued until 24 hours and returned to preoperative levels on postoperative 3rd day. Significant reduction was also observed in coronary sinus blood sample after cardiopulmonary bypass. Decreased Cp levels in blood samples taken before and after cardiopulmonary bypass, except coronary sinus, indicate that cardiopulmonary bypass increases oxidative stress in all organ systems. Significant decrease in the level of Cp enzymatic activity in the coronary sinus blood before and after cardiopulmonary bypass showed reduction in the local myocardial antioxidant levels due to consumption as a respond to oxidant stress. This decline is indicative of an increase in oxidative stress on the heart due to cardiopulmonary bypass. These results suggest that Cp may play an important role in the evaluation of the oxidative stress of the heart. In addition, normalization of Cp levels on postoperative 3rd day revealed that the destructive effect of cardiopulmonary bypass disappeared and the antioxidant level returned to normal. This indicates that the patients undergoing cardiopulmonary bypass should be monitored carefully until the third day of operation.

In conclusion, enzymatic activity of the Cp is a parameter that can be important in determination of antioxidant capacity in cardiovascular procedures especially in coronary artery bypass graft operations. Further studies with large number of patient population are required to improve our knowledge on this subject.

The study was approved by the Clinical Studies Ethical Committee of Harran University by the decision no 02-12-2010-06.

Written informed consent was obtained from patients who participated in this study.

There are no conflicts of interest.
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