Oxidative stress and inflammatory response increase during coronary artery bypass grafting with extracorporeal circulation

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Oxidative stress and inflammatory response increase during coronary artery bypass grafting with extracorporeal circulation

Estresse oxidativo e resposta inflamatória aumentam durante cirurgia de revascularização miocárdica com circulação extracorpórea

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Abstract

Introduction: **Thiobarbituric acid-reactive substance is a marker of oxidative stress. It has cytotoxic and genotoxic actions. C- reactive protein is used to evaluate the acute phase of inflammatory response.**

Objectives: **To assess the thiobarbituric acid-reactive substance and C-reactive protein levels during extracorporeal circulation in patients submitted to cardiopulmonary bypass.**

Methods: **Twenty-five consecutive surgical patients (16 men and nine women; mean age 61.2 ± 9.7 years) with severe coronary artery disease diagnosed by angiography scheduled for myocardial revascularization surgery with extracorporeal circulation were selected. Blood samples were collected immediately before initializing extracorporeal circulation, T0; in 10 minutes, T10; and in 30 minutes, T30.**

Results: **The thiobarbituric acid-reactive substance levels increased after extracorporeal circulation (*P*=0.001), with average values in T0=1.5 ± 0.07; in T10=5.54 ± 0.35; and in T30=3.36 ± 0.29 mmoles/mg of serum protein. The C-reactive protein levels in T0 were negative in all samples; in T10 average was 0.96 ± 0.7 mg/dl; and in T30 average was 0.99 ± 0.76 mg/dl. There were no significant differences between the dosages in T10 and T30 (*P*=0.83).**

Conclusions: **C-reactive protein and thiobarbituric acid-reactive substance plasma levels progressively increased during extracorporeal circulation, with maximum values of thiobarbituric acid-reactive substance at 10 min and of C-reactive protein at 30 min. It suggests that there are an inflammatory response and oxidative stress during extracorporeal circulation.**

Descriptors: **Myocardial revascularization. Coronary artery bypass. Systemic inflammatory response syndrome. Inflammation. Inflammation mediators.**

Resumo

Introdução: **Substâncias reativas do ácido tiobarbitúrico são um marcador de estresse oxidativo. A proteína C reativa é usada para avaliar a fase aguda da resposta inflamatória.**

Objetivos: **Avaliar os níveis de substâncias reativas do ácido tiobarbitúrico e da proteína C reativa durante a circulação extracorpórea em pacientes submetidos à cirurgia de revascularização miocárdica.**

Métodos: **Vinte e cinco pacientes consecutivos (16 homens e nove mulheres com idade média de 61,2 ± 9,7 anos) com doença arterial coronária severa diagnosticada por**

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Abbreviations, acronyms and symbols	
CRP	C- reactive protein
EC	Extracorporeal circulation
SD	standard deviation
TBARS	thiobarbituric acid-reactive substance

angiografia, escalados para cirurgia de revascularização miocárdica com circulação extracorpórea, foram selecionados. Amostras sanguíneas foram coletadas imediatamente antes de iniciar a circulação extracorpórea (T0), 10 minutos após (T10) e 30 minutos após (T30).

Resultados: **Os níveis de substâncias reativas do ácido tiobarbitúrico aumentaram após a extracorpórea (*P*=0,001) com valores médios de 1,5 ± 0,07 em T0; 5,54 ± 0,35 em T10 e 3,36 ± 0,29 mmoles/mg de proteína sérica em T30. Os níveis de proteína C reativa foram negativos em T0 em todas as amostras. Em T10, os valores médios foram de 0,96 ± 0,7 mg/dl e em T30 os valores médios foram de 0,99 ± 0,76 mg/dl. Não houve diferença significativa entre os valores de proteína C reativa nos tempos T10 e T30 (*P*= 0,83).**

Conclusões: **Os níveis de substâncias reativas do ácido tiobarbitúrico e da proteína C reativa aumentam durante a circulação extracorpórea, com máximos valores de substâncias reativas do ácido tiobarbitúrico em 10 minutos e de proteína C reativa em 30 minutos. Estes achados sugerem resposta inflamatória e estresse oxidativo durante a circulação extracorpórea.**

Descritores: **Revascularização miocárdica. Ponte de artéria coronária. Síndrome de resposta inflamatória sistêmica. Inflamação. Mediadores da inflamação.**

INTRODUCTION

Extracorporeal circulation (EC) of blood during cardiopulmonary bypass has been shown to induce the production of several pro-inflammatory molecules such as cytokines, chemokines, growth factors, and vasoactive substances. The ensuing systemic inflammatory response and the super-imposed period of ischemia-reperfusion are conditions that promote the production of oxygen-derived free radical species, which are able to initiate lipid peroxidation and a chain of events leading to cell membrane damage, tissue injury, and functional impairment [1]. One of these complications is the ischemia-reperfusion injury that causes several damages to the myocardium and contributes to the mortality and failure of cardiopulmonary bypass. [2].

To predict and treat these syndromes is a goal in intensive care units. Thiobarbituric acid-reactive substance (TBARS) is a marker of oxidative stress and has cytotoxic and genotoxic actions [3-6]. C- reactive protein (CRP) is used to evaluate the acute phase of inflammatory response [7]. Therefore, we conducted this study to assess the TBARS and CRP levels during EC in patients submitted to cardiopulmonary bypass.

METHODS

Patients

Two-hundred-seventy-six consecutive surgical patients with severe coronary artery disease diagnosed by angiography scheduled for myocardial revascularization surgery with extracorporeal circulation were selected. The study was approved by local Ethical Committee. Written informed consent was obtained from all patients to participate in the study. Exclusion criteria were: (1) diabetes mellitus; (2) myocardial infarction in the last 6 months; (3) ejection fraction < 50%; (4) creatinine blood level > 1.2 mg/dl; and (5) smoking. Therefore, the study was conducted on remaining 25 patients (16 men and nine women; mean age 61.2 ± 9.7 years).

Blood sample collection

Blood samples were collected immediately before initializing EC, $T_{0;}$ in 10 minutes, $T_{10;}$ and in 30 minutes, T_{30}. Plasma TBARS levels were measured according to the method of Buege & Aust [8] and Lapenna et al. [9,10]. Briefly, 0.5 ml of ethylene diamine tetra-acetic acid plasma was added to a reaction mixture (1.0 ml) formed by equal parts of 15% trichloroacetic acid, 0.25 N hydrochloric acid, and 0.375% thiobarbituric acid, plus 2.5 mM butylated hydroxytoluene and 0.1 ml of 8.1% sodium dodecyl sulfate, followed by 30 min heating at 95ºC; pH value of the analytical reaction mixture was about 0.9. Butylated hydroxytoluene was used to prevent lipid peroxidation during heating. After cooling, the chromogen was extracted with n-butanol and read spectrophotometrically at 532 nM. C Reactive Protein (Bioclin®, High-sensitive C Reactive Protein K079) was measured by autoanalyzer equipment (Selectra®).

Statistical analysis

Variables were expressed as mean ± SD and medians. To compare results between times $T_{0,}$ $T_{10,}$ and T_{30} Anova one-way analysis of variance and Student's *t*-test were used. Statistical significance was indicated by a value of $P < 0.05$.

RESULTS

Patient's characteristics are disposed in Table 1. The mean EC time was 63.88 ± 20.75 minutes, whereas from the 25 patients, 13 had an EC time of less than 70 minutes and 12 patients had an EC time more than 70 minutes with a maximum time of 95 minutes. The TBARS levels increased after EC (*P*=0.001), with average values in T_0=1.5 ± 0.07; in T_{10}=5.54 ± 0.35; and in T_{30}=3.36 ± 0.28 mmoles/mg of serum protein (Figure 1).

Table 1. Patients' characteristics

Variable	
Sex (male/female)	16/9
Age (years)	61.16 ± 9.73
EC time (min)	63.88 ± 20.75
Aortic clamp time (min)	47.64 ± 20.07
Ejection fraction (min)	62.32 ± 7.63
Creatinine level (mg/dl)	1.07 ± 0.11

Age, Extracorporeal (EC) time, aortic clamp time, ejection fraction, and creatinine levels are expressed in means±sd

The CRP levels in T_0 were negative in all samples; in T_{10} average was 0.96 ± 0.67 mg/dl; and in T_{30} average was 0.99 ± 0.76 mg/dl. There were no significant differences between the dosages in T_{10} and T_{30} (*P*=0.83; Figure 2). There were no significant differences between TBARS and CRP levels according to EC time (less and more than 70 minutes) in the 3 times of sampling.

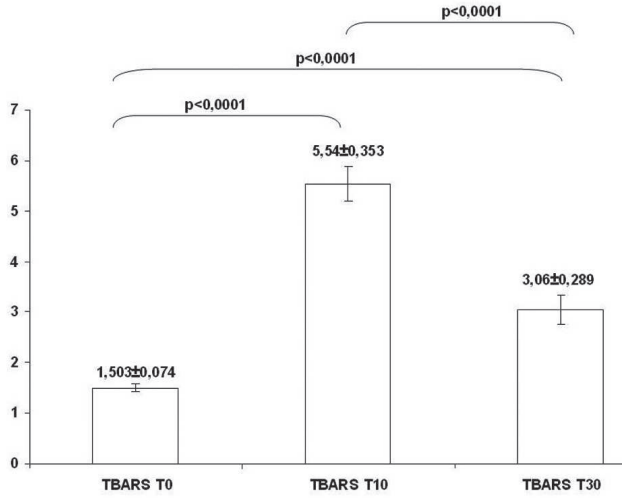

Fig 1. Medians and standard deviations of the doses of TBARS (mmoles/mg of serum protein) in the 25 blood samples collected at T_0, T_{10} and T_{30}

DISCUSSION

Cardiac surgery with cardiopulmonary bypass evokes a systemic inflammatory response syndrome in practically all patients. The intensity of such syndrome experienced by an individual patient will be critical to determine the outcome. To predict the occurrence of postperfusion syndrome during cardiac surgery with EC is a daily real challenge in intensive care units. In the present study we assessed the TBARS and CRP levels during EC in patients submitted to surgical myocardial revascularization. We preselected 276 patients. The initial sample was reduced to 25 subjects, representing only 0.05% of the total population. This fact is due to the rigidity imposed on the inclusion and exclusion criteria, thereby, reducing the chance of any influence of others pathologies on the levels of TBARS and CRP.

Previous studies [11,12] discuss the effects of diabetes on organic reactions in various organs, thus demonstrating that the inclusion of diabetic patients could influence the levels of TBARS and CRP during EC due to underlying disease. Like diabetes, Lucchi et al. [13] demonstrated the correlation between creatinine clearance and increased oxidative stress in tissues and plasma serum. Thereby, we do not included patients with serum creatinine above 1.2 mg/dl as it could act as a factor that changes the results. Smokers were excluded from the sample because they have increased basal levels of TBARS and CRP. Recent data also support a contributory role for reactive oxygen species in the pathophysiology of cardiac hypertrophy and cardiomyophathies and because of that we excluded patients with ejection fraction less than 50% [14].

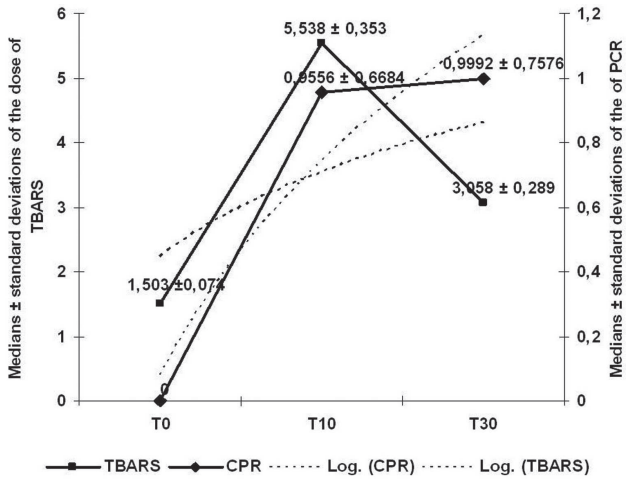

Fig 2. Simultaneous demonstration of the means and standard deviations of the concentrations of TBARS and CRP at T_0, T_{10} and and T_{30}

Measurements of TBARS in three different moments during EC showed significant difference from baseline through T_{30}. It is in agreement with Zanoni et al. [15] that demonstrated the occurrence of immune and inflammatory changes in patients during cardiac surgery with EC. Actually, EC is a nonphysiologic condition imposed to the body, causing cellular structural and functional damage. It triggers a significant inflammatory response in cardiac surgery and is observed that this response is significant lower when the time of EC is less than 70 minutes [16-19].

In the present study, the mean EC time was 63.88 ± 20.75 minutes, whereas from the 25 patients, 13 had an EC time of less than 70 minutes and 12 patients had a EC time more than 70 minutes with a maximum time of 95 minutes. No complication occurred during and after the surgery in any patient. When the results of TBARS and CRP levels were analyzed in two groups according to EC time (less and more than 70 minutes) there were no significant differences between the averages in the 3 times of sampling. The perfusion is related to activation of an inflammatory response that leads to changes in cellular and humoral activation of the complement system and coagulation cascade, causing changes in permeability and vascular reactivity [17-19].

This systemic inflammatory response to cardiopulmonary-bypass has the potential of engendering a constellation of clinical, biochemical, and radiological manifestations of multiorgan dysfunction [20]. Previous studies related neurologic disorders observed in EC, especially in prolonged times [16,21]. In the heart, the ischemia-reperfusion injury includes a series of events: (a) reperfusion arrhythmias, (b) microvascular damage, (c) myocardial stunning 'reversible mechanical dysfunction' and (d) cell death, which may occur together or separately [22]. These changes are considered to be the consequence of imbalance between the formation of oxidants and the availability of endogenous antioxidants in the heart. Maulik et al. [21] demonstrated, in swine, that the oxidative stress developed in the reperfused heart is one of the causative factors for the development of apoptosis. The EC was an important technological development for cardiac surgery, but its safety is not negligible due the inflammatory response generated, and the longer the duration of EC largest degree of aggression is generated in patients, functioning as an independent predictor for postoperative complications.

The use of CRP as an acute inflammatory process marker showed that the dosages at baseline (T_0) were negative and underwent to an increase in T_{10} that remained until $T_{30.}$ Pepys et al. [23] and Volanakis et al. [24] demonstrated in their studies that the increase of CRP in T_{10} and the maintenance in T_{30} is due to the inflammatory stimulus generated by EC that can remain for an average of 19 hours after the activation of the inflammatory process. This was well demonstrated by Milei et al. [25] in 24 patients submitted to myocardial biopsy during cardiac surgery with EC. Because there was a significant elevation of CRP during EC we can say that there is an inflammatory process triggered. However, as CRP remains higher throughout the EC, it is difficult to use it as an isolated marker in the postoperative period. In the present study, we tried to correlate TBARS and CRP levels. It was observed a linear regression in T_{10} that did not remain until $T_{30.}$ Thus, TBARS and CRP levels could not be directly correlated during monitoring patient.

We believe that these are no expensive measurements and their assessment during peri and postoperative period can add new information about the inflammatory response during extracorporeal circulation. One of the limitations of the present study is the small number of patients enrolled. However, without any condition that could interfere in the results, we established the presence of an inflammatory response and oxidative stress during EC. Others studies should be conducted in patients with renal failure, diabetes or smoking habits and compared the levels of TBARS and CRP in different times of EC. The routine measurement of TBARS and CRP during EC can generate different levels of inflammatory response and predict complications during the postoperative period of cardiac surgeries. The results can help to change therapies before a possible complication, such as the early use of corticosteroids and acetylcysteine [15-17].

CONCLUSIONS

C-reactive protein and TBARS plasma levels progressively increased during extracorporeal circulation, with maximum values of TBARS at 10 min and of CRP at 30 min. It suggests that there are an inflammatory response and oxidative stress during EC.

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