Serum Levels of Interleukin-6 (IL-6), Interleukin-18 (IL-18) and C-Reactive Protein (CRP) in Patients with Type-2 Diabetes and Acute Coronary Syndrome without ST-Segment Elevation

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Summary

Background: Atherosclerosis is an inflammatory disease, and serum levels of inflammatory markers such as interleukin 6 (IL-6), interleukin 18 (IL-18) and C-reactive protein (CRP) are used to evaluate patients with coronary artery disease. In patients with type-2 diabetes, atherosclerosis is related to a larger number of events such as myocardial infarction and death, when compared with patients without diabetes.

Objective: To evaluate the inflammatory response in patients with diabetes and acute events of coronary instability.

Methods: Two groups of patients were primarily selected. The first group was comprised of diabetic outpatients with stable angina (D-CCS) and presence of coronary artery disease on coronary angiography (n=36). The second group was comprised of diabetic patients seen in the emergency room with acute coronary syndrome (D-ACS) without ST-segment elevation (n=38). Non-diabetic patients with ACS (n=22) and CCS (n=16) comprised the control group. Serum levels of CRP, IL-6 and IL-18 were determined using nephelometry (CRP) and ELISA (IL-6 and IL-18) techniques.

Results: Higher serum IL-6 levels were found in diabetic or non-diabetic patients with ACS than in the group with CCS. On the other hand, diabetic patients with ACS had higher CRP levels in comparison with the other groups. Serum IL-18 levels were not significantly different among the patients studied.

Conclusion: Our findings suggest a more intense inflammatory activity in patients with coronary instability. This inflammatory activity, as measured by CRP, seems to be even more intense in diabetic patients. (Arq Bras Cardiol 2008; 90(2):86-90)

Key words: Interleukin-6; interleukin-18; coronary arteriosclerosis; diabetes mellitus.

Introduction

Coronary artery disease affects an alarming number of individuals worldwide, side by side with another condition of global proportions: one hundred million people are diagnosed with diabetes mellitus (DM) worldwide[1], 90% of whom have type-2 DM. The incidence of type-2 DM is expected to increase, following the increase in the number of cases of obesity worldwide[2]. Atherosclerosis is the major cause of premature death in patients with type-2 DM, and nearly 80% of all deaths and 75% of all hospital admissions result from complications of atherosclerosis[3].

Inflammation plays a key role in atherosclerosis, whose complications have drawn the attention of researchers and health entities[4]. IL-6 is a cytokine that acts both in the innate and in the adaptive immune response. It is synthesized by monocytes, endothelial cells, fibroblasts and other cells, in response to microorganisms and also to the stimulation by other cytokines, mainly interleukin-1 (IL-1) and TNF-α. IL-18, a recently described cytokine from the IL-1 family, has a pleiotropic action, and participates in the innate as well as in the acquired immune response. Among its main functions is the stimulation of INF-γ production by T lymphocytes, NK cells and macrophages[5,6]. CRP is very mildly elevated in atherosclerosis, requiring the use of highly sensitive methods for its quantification. In the Honolulu Heart Program, the increased risk for acute myocardial infarction (AMI) related to CRP has remained valid even after 20 years[7].

Hyperglycemia itself, a characteristic of glucose intolerance, is related to the immediate synthesis of markers such as IL-6 and IL-18, with serum level variations positively correlated and with more significant increases in hyperglycemic spikes, a situation that is common in diabetic patients[8]. Since patients with diabetes comprise a significant part of the population with coronary artery disease (20-24%), the understanding of the inflammatory mechanisms in diabetes and also in insulin resistance is fundamental for a proper treatment. The objectives of the present study were to analyze the serum levels

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Manuscript received February 17, 2007; revised manuscript received August 11, 2007; accepted October 16, 2007.

of IL-6, IL-18 and hs-CRP in patients with chronic coronary syndrome (CCS) and acute coronary syndrome without ST-segment elevation (ACS), and to compare possible variations of serum levels between the groups according to the presence of diabetes.

Subjects

Adult men and women aged between 40 and 80 years with a previous diagnosis of diabetes, and who were admitted to the emergency room with a diagnosis of acute coronary syndrome (D-ACS) (n=38) or attended the outpatient service of *Hospital das Clínicas* with chronic coronary syndrome (D-CCS) (n=36) were included in the study. The control group was comprised of non-diabetic patients with ACS (n=22) or CCS (n=16). The presence of ACS was defined by a history of at least one episode of angina at rest lasting more than 20 minutes in the preceding 24 hours, with suggestive electrocardiographic changes (ST-segment depression – 1.0 mm in contiguous leads and/or T wave inversion greater than 0.3mm in two contiguous leads). The second group was comprised of patients with stable angina (CCS) in the past six months with signs of coronary artery disease as demonstrated by coronary angiography. The study was approved by the Research Ethics Committee of the School of Medical Sciences of *Universidade Estadual de Campinas*, and a written informed consent was obtained from all participants.

Exclusion criteria

a) Pregnancy;

b) Class III or IV congestive heart failure;

c) Valvular heart disease;

d) History of acute myocardial infarction in the preceding four weeks;

e) Atrial fibrillation;

f) Any ECG abnormality that could affect the analysis of the ST-segment;

g) History of surgery or trauma in the preceding four weeks;

h) History of chronic or acute inflammatory disease;

i) History of malignancies;

j) Acute myocardial infarction as defined by laboratory criteria (CKmb elevation greater than two-times the cut-off value).

Methods

After selection and enrollment with an informed consent form, the patients had their venous blood drawn for determination of serum levels of IL-6, IL-18, high-sensitivity C-reactive protein, and markers of myocardial necrosis. Blood was also used for determinations of blood count, coagulation tests, urea, creatinine, sodium, potassium, blood glucose, and lipid profile. No additional venous puncture was performed in unstable patients, since punctures are performed as a routine diagnostic procedure. All patients received the usual treatment and no medication was added or discontinued by study intervention. Unstable patients were closely monitored in the emergency room or coronary unit. Patients could drop from the study at any time they wished. The samples were centrifuged and kept at -80°C for further analysis. The mean time elapsed from the last episode of pain and blood collection was 14 hours.

Serum levels of IL-6 and IL-18 were determined using the ELISA technique, with the R&D Systems (Minneapolis, MN, USA) and MBL-Medical & Biological Laboratories (Nagoya, Japan) commercial kits, respectively, according to the manufacturer's instructions. In this technique, a specific monoclonal antibody is adsorbed onto a plate. After addition of the serum sample where the mediator to be determined is placed, the material is incubated, and this is the moment when the antigen molecules will bind to the antibodies adsorbed onto the plate. All unbound material is washed away. Next, a new antibody specific for an antigenic determinant linked to the plate is added, and an Ab-Ag-Ab-enzyme complex is obtained (sandwich technique). The material is washed again to remove the unbound antibodies. Then, a substrate with the property of turning into a different color when in contact with the enzyme is added in proportion to the amount of the mediator present in the sample (antigen). The reading is performed in a plate reader (BioRad, Tokyo, Japan) at 450 nm and compared to a standard curve obtained with known concentrations of the recombinant mediators. The detection limits of the essays were: 0.09 pg/ml for IL-6, and 12.5 pg/ml for IL-18.

Hs-CRP levels were determined using the nephelometry technique in a BNII analyzer (Dade Behring, Marburg, Germany). The reagent consists of a polystyrene particle suspension coated with anti-CRP monoclonal antibody which agglutinates in the presence of CRP in the sample. The intensity of the scattered light in the nephelometer depends on the concentration of CRP in the sample, so that, through comparison with dilutions of a standard of known concentrations, it is possible to determine the concentration of this mediator in the samples. The limit of the technique is 0.175 mg/l.

Statistical analysis

Specific variables

Clinical variables - Age, gender, history of AMI, previous myocardial revascularization, previous angioplasty, hypertension, smoking, history of dyslipidemia, family history of early coronary artery disease, use of nitrates, beta blockers, calcium antagonists, aspirin, statins, angiotensin converting enzyme inhibitors and diuretics.

Laboratory variables - blood count, IL-6, IL-18, CRP, CK, CKMB, LDH, AST, urea, creatinine, sodium, potassium, blood glucose, LDL, HDL, triglycerides and total cholesterol.

Primary endpoints - IL-6, IL-18 and CRP levels.

Statistical methods - All data were inserted in a Microsoft Access database table. Non-parametric tests were used for data with a non-normal distribution. Results are expressed as medians. The Mann-Whitney *U* test was used to evaluate the differences between the groups. The Friedmann test was used to evaluate the differences within the same group. The *t* test for non-paired observations was used to analyze continuous variables. Category and proportion data were analyzed using the χ^2 or Fisher test

when required. P values < 0.05 were considered statistically significant. The SPSS 8.0 statistical software package (SPSS Inc) was used for all calculations. The number of patients was based on previous studies[9] considering the values for IL-6.

Results

The clinical characteristics of the groups studied are shown in Table 1. No significant differences were found among the different parameters analyzed, except for the more frequent use of statins in the group of diabetic patients with ACS.

Blood glucose levels were determined without fasting, and varied significantly between the groups with or without diabetes (D-ACS= 186±28 mg/l, D-CCS= 158±59 mg/l, ACS= 92±14 mg/l and CCS= 86±12 mg/l).

Approximately 50% of the patients with ACS had already had an infarction, almost half of them were hypertensive, one-fourth were smokers, more than three-thirds were receiving beta blockers, and almost 90% were receiving aspirin.

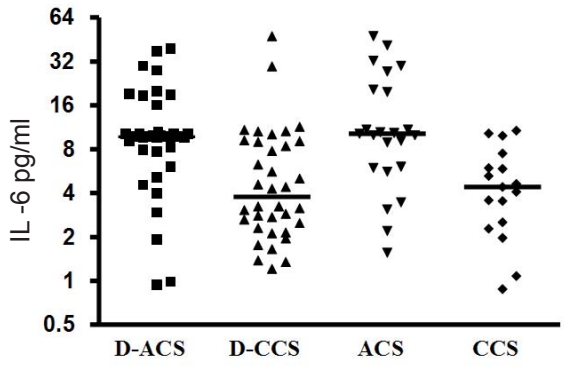
IL-6 showed a statistically significant difference between the groups D-ACS and D-CCS (median of 11.98 pg/ml and 6.858 pg/ml, respectively, p<0.0149), and between the groups ACS and CCS (median of 14.68 pg/ml and 4.971 pg/ml, respectively, p<0.0040) (Graph 1).

The analysis of serum IL-8 levels showed similar levels between the groups D-ACS (median 289.4 pg/ml), D-CCS (median 334.5 pg/ml), ACS (median 281.9 pg/ml) and CCS (median 322.3 pg/ml) (Graph 2).

CRP levels were significant in the comparison between groups D-ACS and D-CCS (median of 4.75 mg/l and 0.75 mg/l, respectively, p< 0.0002), as well as between groups D-ACS and ACS (median of 4.750 mg/l and 1.83 mg/dl, respectively, p=0.0402), and finally between D-ACS and CCS (median of 4.75 mg/l and 0.55 mg/l, respectively, p=0.0062) (Graph 3).

Discussion

The number of adult individuals diagnosed with type-2 DM will rise to approximately three hundred million by 2025.

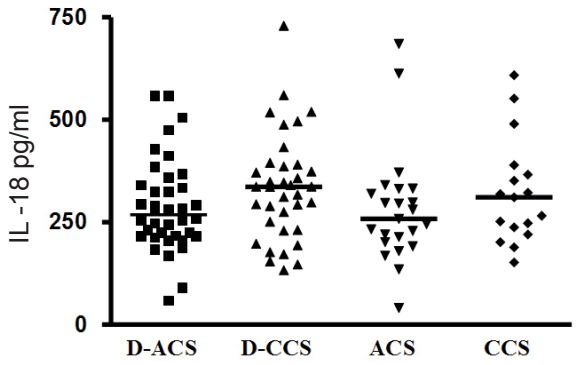


Graph 1 - Serum IL-6 levels in patients with D-ACS (n=38), D-CCS (n=36), ACS (n=22) and CCS (n=16). Horizontal lines represent medians. D-ACS vs D-CCS, p< 0.01. ACS vs CCS, p< 0.004.

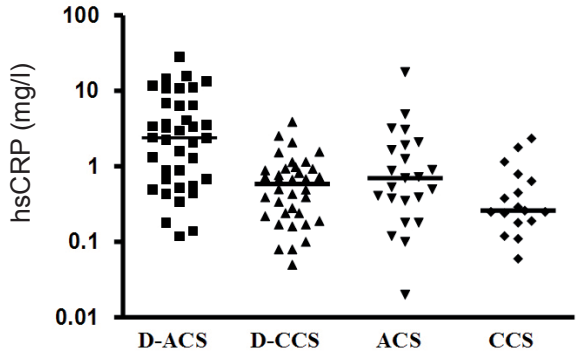
Tabela 1 - Clinical characteristics of the patients enrolled in the study

	D-ACS(38)	D-CCS(36)	ACS(22)	CCS(16)	p
Men/Women, n	38/17	36/14	22/10	16/7	0.26
Age, years	61±9.7	58±10.5	58±9.7	60±12.1	0.46
Hist. AMI, n(%)	20(52)	20(55)	10(48)	8(50)	0.34
Previous Revasc, n(%)	5(13)	8(22)	2(10)	8(20)	0.17
Previous Angi, n(%)	10(26)	8(22)	5(24)	4(25)	0.29
Hypertension, n(%)	17(44)	22(61)	11(50)	8(50)	0.18
Smoking, n(%)	10(26)	12(33)	5(24)	4(25)	0.34
Dyslipidemia, n(%)	29(76)	19(52)	12(54)	8(50)	0.23
Family Hist., n(%)	25(65)	22(61)	10(48)	12(75)	0.62
Nitrates, n(%)	26(68)	19(52)	11(50)	10(60)	0.29
Beta Block, n(%)	33(86)	18(50)	11(50)	12(75)	0.09
Calcium Antag, n(%)	26(68)	18(50)	14(66)	8(50)	0.16
Aspirin, n(%)	34(89)	33(91)	18(81)	15(93)	0.10
Statin, n(%)	29(76)	16(44)	14(66)	10(60)	0.04*
ACEI, n(%)	9(23)	8(22)	5(24)	4(25)	0.41
Diuretic, n(%)	10(26)	12(33)	10(48)	4(25)	0.26
Total Chol., mg/dl	228±19	221±24	210±21	227±19	0.90

*Age expressed as mean ± SD. Other data are expressed as number of individuals (percentage). AMI - myocardial infarction; Revasc. - surgical revascularization; and Angio - percutaneous transluminal angioplasty. * Statistical significance (p < 0.05).*



Graph 2 - Serum IL-18 levels in patients with D-ACS (n=38), D-CCS (n=36), ACS (n=22) and CCS (n=16). Horizontal lines represent medians.



Graph 3 - Serum hsCRP levels in patients with D-ACS (n=38), D-CCS (n=36), ACS (n=22) and CCS (n=16). Horizontal lines represent medians. D-ACS vs D-CCS, p= 0.002. ACS vs CCS, p= 0.04. D-ACS vs CCS, p=0.006. D-ACS vs ACS p=0.04.

These patients will probably have a tendency to a more rapidly progressive atherosclerosis. Using the definition of type-2 DM as an inherited or acquired insulin resistance in the presence of a genetically determined beta-cell failure, we can consider insulin resistance as a key element in type-2 DM. Insulin resistance is associated with a higher risk of coronary artery disease and is present in patients with type-2 DM as well as in patients with metabolic syndrome (hypertriglyceridemia, low HDL levels, hypertension, abdominal obesity and, of course, insulin resistance).

By sharing classic risk factors with atherosclerosis, type-2 DM may also share a common etiologic pathway which can be the inflammatory pathway.

In atherosclerosis, the detection of high levels of inflammatory markers, notably of CRP, is related to a worse outcome of patients with CAD. By lowering cholesterol levels, medications such as statins also result in decreased serum CRP levels, and this reduction seems to be related to a better prognosis[10]. Pleiotropic effects of statins, in addition to the effect of cholesterol lowering, have been demonstrated in several studies and reviews[11]. By acting on several mediators of inflammation, HMG-CoA reductase inhibitors reduce monocyte adhesion to the endothelium, thus reducing migration and proliferation of inflammatory cells; improve endothelial function; reduce extracellular matrix breakdown; and reduce thrombogenic factors and inflammatory cytokines. Clinical studies are being conducted to confirm independent benefits of cholesterol levels lowering.

Our study showed that the use of statins was less frequent in group D-CCS, a finding that could justify the higher CRP levels in this group in comparison with group CCS. However, studies conducted up to the present have shown that statin-related CRP reduction is linked to cholesterol lowering, and cholesterol levels were not significantly different between the groups.

Yudkin et al[12] studied 107 non-diabetic patients and demonstrated that CRP, IL-6 and TNF-α levels are elevated and positively correlated with measurements of insulin resistance. Another study[13] showed that serum CRP levels are linearly correlated with the number of components of the metabolic syndrome. Experimental studies show that IL-6 interferes with insulin synthesis in pancreatic β cells and reduces insulin-stimulated glycogen synthesis in hepatocytes[14].

Atheroma plaques of diabetic patients with coronary syndrome exhibit a larger content of lipid, thrombosis and macrophage infiltration than the coronary tissue from patients without diabetes[15]. This high proportion of infiltrated inflammatory cells suggests not only that inflammation plays a key role in atherosclerosis, but also that the proinflammatory state is more active in patients with diabetes.

This clear relationship led to the research of other inflammatory markers in type-2 diabetic patients, in the search of the same alteration in relation to non-diabetic patients and also diabetic patients, this latter, however, only in the chronic phase of coronary artery disease. As regards IL-18, our findings did not show significant differences between the different groups, unlike several retrospective studies. The prospective design of this study confers greater value to the data presented here . However, some aspects of serum determination of an unstable marker, as is IL-18, may have contributed to this finding, since it is a cytokine that is certainly involved in plaque instability and increased local concentrations. However, the absence of increased serum levels verified is contrary to the therapeutic perspectives involving IL-18 receptor blockade.

As has already been demonstrated in previous studies, IL-6 is significantly increased in acute manifestations of coronary instability, and our study corroborates the role of this cytokine in the setting of atherosclerotic plaque rupture or erosion. This increase may be related to CRP elevation, since IL-6 promotes the hepatic synthesis of this marker. However, CRP also has its atherogenic effect, partly mediated by IL-6 synthesis[16].

The most original finding of the present study was related to CRP. Our study demonstrated six-fold higher serum CRP levels in diabetic patients with acute coronary artery disease in comparison with diabetic patients with chronic coronary artery disease. The serum CRP level in the group of diabetic patients with ACS without ST-segment elevation was 4.5 mg/dL, thus exceeding by 50% the basic level for high risk as defined by the American College of Cardiology (2002) for this marker. When we

focused on the groups with ACS with and without diabetes, CRP was still 2.6-fold higher in diabetic patients. Initially described as a marker of the inflammatory response, an active role of CRP as a mediator of atherogenesis has been demonstrated in recent studies. CRP induces apoptosis of endothelial cells, inhibits angiogenesis and stimulates transcription of several genes of proinflammatory cytokines[17]. On the other hand, it stimulates endothelin-1 and IL-16 release, leading to an increased expression of adhesion molecules and chemokines involved in the recruitment of inflammatory cells such as MCP-1. Thus, we can presume that the increased CRP levels in diabetic patients with ACS represent a high inflammatory state and damage. There was also a trend (p=0.08) of increased CRP levels in diabetic patients with chronic coronary disease when compared with non-diabetic patients with chronic coronary disease, thus signaling a high inflammatory activity in diabetic patients even in the stable phase of coronary artery disease. The analysis of the present study was limited to the patients' baseline findings, and the patients are being clinically followed up so that information on prognosis in the groups studied can be obtained. The time of sample collection, specifically in unstable patients, does not seem to interfere with the results obtained[18]. The results regarding blood glucose found in the group D-CCS may be questioned as a factor leading to CRP elevation in relation to group CCS. A study showed elevation of these two variables in diabetic patients[19], and this is in agreement with the pathophysiology of the hyperglycemia-induced oxidative stress. However, the present study could only demonstrate, by means of CRP, that inflammation is increased in this group of patients, who are frequently found in a hyperglycemic state (D-CCS = 158±59mg/l).

Conclusions

Our study showed that the inflammatory reaction of atherosclerosis in diabetic patients, mediated by hsCRP, is more intense in patients with acute coronary disease when compared both to diabetic patients with chronic coronary disease and to non-diabetic patients with acute coronary disease. This finding partly explains the worst prognosis of patients with diabetes and coronary artery disease, and implies that greater efforts should be made toward the control of inflammation and improvement of survival of this significant part of the population.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by Fapesp.

Study Association

This article is part of the thesis of master submitted by Roberto Matos Souza, from *Departamento de Clínica Médica da Faculdade de Ciências Médicas da Unicamp*.

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