Cellular Immunology 310 (2016) 178–183

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

Serum interleukin-6: Association with circulating cytokine serum levels in patients with sinus arrhythmia and patients with coronary artery disease

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ARTICLE INFO

Article history:
Received 10 April 2016
Revised 28 July 2016
Accepted 8 September 2016
Available online 9 September 2016

Keywords:
Cytokines
Heart
Sinus arrhythmia
Interleukin-6

ABSTRACT

In this study, we were focused on the differences between certain circulating cytokine levels in patients with or without sinus arrhythmia, according to the median IL-6 level. All patients were stable with regards to symptoms and therapy for at least one month prior to the measurements conducted within this study. Exclusion criteria were: patients with sleep apnea, asthma, respiratory insufficiency of any genesis, active infection, allergy, inflammatory diseases, cancer, diabetes of any type and treatment with anti-inflammatory drugs. The study was approved by the Institutional Review Board. All recruited patients gave their verbal and written consent for participation in the study. The study group consisted of 74 patients divided into two groups: with (38) and without sinus arrhythmia but with diagnosed coronary artery disease (36). Sinus arrhythmia was confirmed by 24 h Holter monitoring. From all test parameters only cytokines IL-2, IL-8, IL-10, IL-17 and IL-18, showed statistically significant increasing in patients with statistically higher IL-6 levels. It is possible that IL-6 may not be a marker for the selection of patients with sinus arrhythmia or coronary artery disease. The findings indicate that IL-6 represents a reliable indicator for increased expression of IL-2, IL-8, IL-10, IL-17 and IL-18 in patients with sinus arrhythmia or coronary artery disease. Further studies in a large number of patients would be necessary to confirm our observations.

1. Introduction

The mechanisms underlying increased immunogenicity of body proteins in the cardiovascular system, the roles of immunogenic auto-antigens in eliciting inflammatory autoimmune responses, and the immunosuppressive mechanisms involved in controlling inflammatory and autoimmune cardiovascular diseases remain to be well-understood [1]. A significant association of inflammation indicating increased concentration of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), along with heavy meals, decrease in magnesium, potassium, coenzyme Q10 and nitrite in relation to circadian rhythms with risk of acute coronary syndromes have been commonly observed [2]. These findings pose a possibility that rhythm disturbances of any kind may be associated with increase in cytokines. An increased concentration of cytokines has also been observed among patients of chronic heart failure which may be associated with arrhythmogenesis and class III and IV heart failure compared to class I and II heart failure [3]. Chronic heart failure may be associated with neurohumoral dysfunction characterized with increased concentrations of catecholamines, cortisol and angiotensin II which may cause oxidative stress and inflammation and promote the loss of myocytes by apoptosis leading to rhythm disturbances [4].

Abbreviations: LVEF, left ventricular ejection fraction; LAV, left atrial volume; PASP, pulmonary artery systolic pressure; LVSV, left ventricular stroke volume; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; BPM, beats per minute; RAV, right atrial volume; SA, sinus arrhythmia; CAD, coronary artery disease.

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http://dx.doi.org/10.1016/j.cellimm.2016.09.007
0008-8749/

IL-6 as a pleiotropic cytokine, functions as a mediator of inflammatory response and has both, pro- and anti-inflammatory properties. IL-6 has been identified as a differentiation factor of B and T-lymphocytes, hepatocytes, neuronal cells, and hemopoetic precursors [5]. Humphries et al. [6], reported that IL-6 was involved in the pathogenesis of cardiovascular disorders. Additional studies demonstrated that IL-6-174 G/C polymorphisms are associated with higher serum levels of IL-6, risk of coronary heart disease, acute coronary syndrome and atrial fibrillation [7].

Serum levels of IL-6 higher than 20 pg/ml in the first 24 h after ST-segment elevation at acute myocardial infarction are significantly associated with the higher frequency of in-hospital onset such is death [8]. Significant relationships between preoperative IL-6 and atrial fibrillation in patients who underwent an on-pump coronary artery bypass grafting was also reported [9]. According Ucar et al. [10], elevated IL-6 and highly sensitive C-reactive protein (hsCRP) in patients with postoperative atrial fibrillation, suggests that inflammatory components play very important roles in the pathogenesis of atrial fibrillation. Also, reduction of long-term heart rate variability is associated with increased levels of IL-6 in patients with de-compensated heart failure [11].

The IL-6 level provides prognostic information that are complementary to clinical risk scores for the prediction of long-term cardiovascular events and death, suggesting that this bio-marker may potentially be used to refine clinical risk stratification in atrial fibrillation [12]. Elevated IL-6 serum concentrations were prospectively associated with an increased risk of spontaneous ventricular tachyarrhythmia in patients with an implantable cardioverter-defibrillator. These preliminary findings support a possible association of pro-inflammatory activity and an increased susceptibility to spontaneous ventricular tachyarrhythmia in the same patients [13].

All above studies reported a positive association between increased circulatory IL-6 concentration and different heart pathologies. Despite growing body of data in the field, it is unclear up to date whether elevated circulatory IL-6 plays an important role in patients with sinus arrhythmia (SA). Consequently, the objectives of our study were: to find out the role of IL-6 in patients with SA and in those without arrhythmia but with coronary artery disease (CAD); and to correlate the association of IL-6 levels with other subclasses of cytokines in the studied groups of patients.

2. Materials and methods

2.1. Patient data

This was a single-center prospective study carried out between 2014 and 2015 in 74 consecutive patients with SA ($n = 38$) and without SA but with diagnosed CAD ($n = 36$). Patients were treated according to the guidelines of the American Heart Association/American College of Cardiology. The study conformed to the principles outlined in the Helsinki Declaration and was approved by the Institutional Review Board (Ethics Committee of the Russian National Research Medical University N.I. Pirogov). After receiving appropriate information all recruited patients gave their verbal and written consent for participation in the study.

SA was confirmed by 24 h Holter monitoring, instituted the same day when an echo-cardiogram had been evaluated and blood samples taken for biochemical analysis. SA included sinus node dysfunction (SND) ($n = 22$), bradycardia ($n = 7$) and tachycardia ($n = 9$). The study group consisted of 46 male and 28 female patients. As the study was designed to avoid mixing entities that are known to have a very different prognosis only patients with heart rate dysfunction and CAD were included, while patients with sleep apnea, asthma, respiratory insufficiency of any genesis, active infection, allergy, inflammatory diseases, cancer, diabetes of any type and treatment with anti-inflammatory drugs were excluded.

Table 1
Study patient characteristics.

Medications	
β-blockers (*n*, %)	43(57.3%)
Hypolipidemic agents (*n*, %)	51(68.0%)
Diuretics (*n*, %)	13(17.3%)
Proton-pump inhibitor (*n*, %)	19(25.3%)
ACE inhibitor (*n*, %)	26(34.7%)
Antiplatelet drug (*n*, %)	32(42.7%)
Antiarrhythmic agents (*n*, %)	13(17.3%)
Anticoagulants (*n*, %)	29(38.7%)
Calcium channel blockers (*n*, %)	26(34.7%)
Diseases	
Systemic hypertension (*n*, %)	48(64%)
Chronic renal failure (*n*, %)	0 1(1.33%)
Systolic blood pressure (mmHg ± SD)	0 135.96 ± 19.07

All patients were stable with regards to symptoms and therapy for at least one month prior to the measurements conducted within this study. The patients were on standard medication consisting of β-blockers (57.3%), hypolipidemic agents (68%), diuretics (17.3%), proton-pump inhibitor (25.3%), ACE inhibitor (34.66%), antiplatelet drug (42.66%), antiarrhythmic agents (17.33%), anticoagulants (38.66%) and calcium channel blockers (34.66%). History of systemic hypertension (64%), chronic renal failure (1.33%) and systolic blood pressure were also assessed (Table 1).

2.2. Biochemical analysis

Venous blood samples were taken from the patient's ulnar vein, between 8.00 and 9.00 a.m. for biochemical tests after overnight fasting, using vials without any anticoagulant. Serum was prepared by centrifugation (2000*g* for 20 min.), separated into aliquots and stored at −80 °C until analyses were performed. Serum samples were analysed for: sodium (Na), glucose (Glu), creatinine (CRT), triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and urea (U) by an Automatic biochemical Analyzer (Abbott Architect c8000 Chemistry Analyzer).

2.3. IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-18 and VEGF-immunoassays

IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-18 and VEGF in the serum were analysed by the newly developed ELISA for quantitative analysis of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-18, and VEGF levels from Bender Med-Systems. The limits of detection of the assays were about 1.0 pg ml^{-1} for IL-1β, 2.0 pg ml^{-1} for IL-2, 0.4 pg ml^{-1} for IL-4, 0.92 pg ml^{-1} for IL-6, 2.0 pg ml^{-1} for IL-8, 1.0 pg ml^{-1} for IL-10, 2.0 pg ml^{-1} for IL-17, 2.0 pg ml^{-1} for IL-18 and 10.0 pg ml^{-1} for VEGF. Inter- and intra-assay CVs were 6.4% and 3.1% for IL-1β, 7.7% and 5.8% for IL-2, 8.3% and 8.6% for IL-4, 5.2% and 3.4% for IL-6, 8.9% and 6.1% for IL-8, 9.8% and 9.4% for IL-10, 4.8% and 8.5% for IL-17, 5.8% and 7.6% for IL-18, and 8.1% and 9.7% for VEGF.

2.4. Instrumental diagnostic methods

Electrocardiograms (ECG) from the patients were recorded before blood sampling. The following time domain variables were computed for each subject: heart rate (HR), PQ interval, QRS complex, QT interval. Echo-cardiograms (Echo-CG) were also recorded before blood sampling measuring the following parameters: left