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Cytokine gene polymorphisms are associated with markers of disease severity and prognosis in patients with idiopathic dilated cardiomyopathy

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ABSTRACT

Aims: To identify potential genetic associations of five cytokine gene polymorphisms with disease severity and prognosis in patients with idiopathic dilated cardiomyopathy (DCM).

Methods and results: Eighty patients with DCM were genotyped for transforming growth factor beta1 (TGF-β1) +869 T/C (codon10 Leu → Pro), TGF-β1 +915 G/C (codon25 Arg → Pro), interleukin (IL)-6 −174G/C, tumor necrosis factor-alpha (TNF-α) −308A/G, interferon-gamma (IFN-γ) +874T/A, IL-10 −1082A/G, IL-10 −819T/C and IL-10 −592A/C gene polymorphisms. In homozygous TT patients for TGF-β1 +869 T/C polymorphism mean VO_2 max was significantly higher than in CC homozygous patients (25.67 ± 6.73 ml/kg/min vs. 20.29 ± 6.35 ml/kg/min, $p = 0.046$), which remained significant only for patients younger than 39 years old after adjusting for age and sex ($p = 0.009$). C carriers of TGF-β1 +915 G/C polymorphism are 4.2 times more likely to be in a worse NYHA stage (III–IV) than non C carriers [OR: 4.25, 95% CI (1.53–11.80), $p = 0.006$]. Patients GG homozygous for IL-6 −174G/C polymorphism presented greater left ventricle end-systolic ($p = 0.018$) and end-diastolic ($p = 0.04$) diameters in comparison to the CC homozygous. The AA homozygote for IFN-γ +874T/A polymorphism ($p = 0.02$) and the combination of the TGF-β1 +869 T/C and TGF-β1 +915 G/C genotypes were associated with adverse outcome ($p = 0.014$).

Conclusion: Specific cytokine gene polymorphisms seem to be associated with worse prognosis as well as with measures of disease severity in DCM.

1. Introduction

Extensive evidence supports the involvement of cytokines in the inflammatory and immune responses mediating the pathogenesis of idiopathic dilated cardiomyopathy (DCM) [1,2]. The genetic contribution of cytokine network to the prevalence and the progression of this disease have been investigated by a number of studies [3–5]. Several polymorphisms of cytokine genes or gene promoters, may affect gene transcription, influencing the *in vivo* and *in vitro* cytokine production [3–5].

Among the major circulating cytokines implicated in the development of the syndrome are the proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and interferon-γ (IFN-γ), which have been found to be upregulated in heart failure [2]. TNF-α and IL-6 have also been shown to be predictors for cardiovascular outcome whereas a strong association has been found between IFN-γ polymorphism and susceptibility to DCM [6,7]. Levels of transforming growth factor-β1 (TGF-β1) – an anti-inflammatory cytokine – are elevated in the circulation of patients with congestive heart failure and have been associated with maladaptive ventricular remodelling [8]. The diagnosis of DCM has been associated with a reduction in IL-10 plasma levels, indicating its protective role in cytokine activation [9].

The present study is a pilot study, addressing the hypothesis that cytokine gene polymorphisms may reflect the severity and progression of DCM, ultimately leading to adverse outcome. In this respect, we examined polymorphisms of the most important inflammatory cytokines-TGF-β1, IL-6, IL-10, TNF-α and IFN-γ in 80 patients with DCM. We investigated whether these gene polymorphisms are associated with patients' symptoms and exercise capacity, as expressed by peak oxygen uptake (VO_2 max) as well as with echocardiographic indexes of cardiac remodelling and contractile performance, as expressed mainly by left ventricular end

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diastolic and end-systolic diameters (LVEDD and LVESD) and the ejection fraction (EF). Furthermore, we investigated whether these cytokine polymorphic variations are associated with prognosis in a long follow-up period.

2. Methods

2.1. Study population

Patients were recruited from consecutive DCM patients referred for evaluation in the Second Department of Cardiology of the Onassis Cardiac Surgery Center between 1999 and 2003. The clinical diagnosis of DCM was confirmed according to the report of the World Health Organization/International Society and Federation of Cardiology Task Force [10]. The exclusion criteria were as follows: (1) history of myocardial infarction, (2) history of alcohol abuse, (3) evidence of coronary artery disease confirmed by diagnostic angiography or valvular heart disease, (4) active myocarditis or post-myocarditic state, and (5) secondary heart muscle diseases. Patients were not included in the study if they had chronic lung disease, chronic renal failure, cancer, or other no cardiac conditions that might limit exercise capacity. All patients enrolled in the study were in clinical stable condition and received conventional therapy for at least 3 months before recruitment.

At baseline, all patients underwent evaluation including New York Heart Association (NYHA) classification, clinical examination, electrocardiogram, M-mode and 2-dimensional echocardiography and cardiopulmonary exercise test. This study was approved by the Ethics Committee of the Onassis Cardiac Surgery Center. All patients provided a written informed consent.

2.2. Echocardiographic examination

Echocardiographic measurements were performed in all patients using a Hewlett–Packard system (Sonos 1000 or 2500; Palo Alto, California). A comprehensive 2D and Doppler echocardiography was performed according to the recommendations of the American Society of Echocardiography [11]. Left ventricular dimensions (LVEDD and LVESD) were measured with M-mode echocardiography by using the left parasternal window. Left ventricular EF was determined by apical two- and four-chamber views with the modified Simpson rule [11].

2.3. Cardiopulmonary exercise test (CPX)

Patients underwent CPX on a treadmill (Medgraphics); the Dargie protocol was used. VO_2 (ml/min), VCO_2 (ml/min), and minute ventilation (L/min) were measured continuously. All patients terminated the exercise because of dyspnoea or fatigue. The gas exchange anaerobic threshold and a respiratory exchange ratio >1.0 were reached in all patients. Peak oxygen consumption (VO_2 max, ml/kg/min) during exercise was reported as the mean value during the last minute of exercise [12].

2.4. Measurement of BNP

During the initial evaluation, peripheral blood samples were obtained from all patients after they had remained in supine position for 30 min. Blood samples were centrifuged within 30 min, at 3000 c/min for 10 min at 4 °C. Plasma was extracted and stored at −70 °C until further analysis. For the measurement of plasma brain natriuretic peptide (BNP) concentrations, a competitive enzyme immunoassay kit was used (Biomedica, Wien, Austria). Inter-assay and intra-assay variabilities were 3.8–4.4% and 4.0–6.5%, respectively.

2.5. Genotypic analysis

Peripheral blood samples were collected from each individual and stored frozen at −20 °C until further analysis. Genomic DNA was extracted using a commercially available kit (QIAGEN, Germany). All individuals were genotyped for 8 polymorphic sites in 5 cytokine genes, namely: TGF-β1 +869 T/C and +915 G/C, IL-6 −174 G/C, TNF-α −308 A/G, IFN-γ +874Τ/Α, IL-10 −1082 A/G, −819 C/T and −592 A/C. Genotyping was performed using a polymerase chain reaction with sequence specific primers (PCR–SSP) assay with a commercially available cytokine genotyping kit (One Lambda, USA). PCR products were run on 2% agarose gels and visualized with ethidium bromide. Banding patterns were interpreted using manufacturer's templates and compared to internal controls in each lane. The reproducibility of the genotype assignment was validated by repeating the DNA extraction process and the genotyping assay for six randomly selected samples from our patient cohort. All genotypes were identical in both runs.

2.6. Patient follow-up

After initial evaluation, patients were scheduled for follow up at three and 6 months and every 6 months thereafter. During the follow up visits, the patients' clinical status was evaluated with regard to heart failure symptoms and functional class changes. The clinical follow-up was performed in a blind manner with respect to patient's genetic status. The end points during follow up were: (1) cardiac death, including death due to pump failure or sudden cardiac death, (2) cardiac transplantation and (3) hospitalisations due to heart failure decompensation.

2.7. Statistical analysis

Genotype, haplotype and phenotype frequencies for all cytokine gene polymorphisms or their combinations were calculated by direct counting. In case of phenotype, the frequencies were obtained by measuring the number of individuals in a population positive for an allele, whereas haplotype frequencies were obtained by measuring the number of chromosomes bearing an allele.

All reported variables are normally distributed except for the EF, which was analyzed after logarithmic transformation was applied. Associations between NYHA stage and genes polymorphisms were investigated using chi-2 test and ordinal logistic regression models whilst for LVEDD, LVESD and VO_2 max, using linear regression models. Test for differences in a continuous marker among XX, YY and XY combinations was performed using regression (ANOVA) models with 2 dummy variables representing the two categories with higher values. Using this parameterization, these categories were compared with baseline category (the one with lowest mean marker value) and significant differences were identified avoiding post hoc tests. Cox proportional hazard models and Kaplan–Meier curves were used to test association with survival time. A p-value <0.05 was considered as statistically significant. Statistically significant results in univariable analysis were further tested in multivariable models, controlling for age and gender.

3. Results

Eighty-three Greek patients who met the inclusion criteria were enrolled to the study. Three of them were lost to follow up. Finally, 80 patients were included in the analysis. The demographic and clinical characteristics of the patients are summarized in Table 1. The genotype frequencies of the studied polymorphisms were similar to those previously published in other Greek populations (Table 2), [13].