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Original article

An inverse correlation between TNF alpha serum levels and heart rate variability in patients with heart failure

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ABSTRACT

Background: Recent evidence indicates that chronic heart failure (CHF) is accompanied by both activation of the immune system and autonomic imbalance. There is a growing body of evidence that increased levels of proinflammatory cytokines and other inflammatory markers have important roles as mediators of disease progression and markers of mortality in patients with CHF.
Objective: The aim of this study was to investigate connection between autonomic imbalance [obtained by analysis of heart rate variability (HRV)] and activation of the immune system [as measured by serum levels of tumor necrosis factor (TNF)-α] in patients with chronic heart failure.
Materials and methods: This cross-sectional study included 21 patients with CHF and 8 age- and gender-matched healthy control subjects. We assessed HRV by 24-hour electrocardiographic Holter monitoring and measured serum levels of TNF-α using an enzyme-linked immunosorbent assay. Clinical assessment and echocardiography were also performed.
Results: There was an inverse correlation between serum level of TNF-α and a time-domain parameter of HRV – SDNN ($r = -0.542$, $p < 0.05$). A similar result was found for HRV triangular index, a geometric measure of HRV ($r = -0.556$; $p < 0.05$). The correlation was stronger for subjects with a diabetes mellitus, females, and TNFA2 allele carriers (an "A" at position −308A). The pNN50, indirect marker of cardiac vagal activity, was not significantly associated with serum concentration of TNF-α.
Conclusions: In conclusion, the results of the present study indicate that increased serum TNF-α level is significantly associated with reduced HRV indices, suggesting that activation of the immune system in patients with CHF is closely related to autonomic imbalance.

Introduction

Activation of the immune system and persistent inflammation are important features of chronic heart failure (CHF), as evidenced by increased circulating levels of pro- and anti-inflammatory cytokines [1–4]. Autonomic imbalance and immune activation are temporally related [5], occurring concomitantly in the early stage of the natural history of CHF [6,7], or in the period of asymptomatic left ventricular dysfunction [8], irrespective of the CHF etiology [9,10]. Only in certain cases, serum levels of cytokines could be etiology or disease-specific [11].

However, mechanisms of immune activation and loss of autonomic balance in CHF are not completely understood. Tracey described the cholinergic anti-inflammatory pathway which controls cytokine production via parasympathetic or vagal nerve activity [12]. Stimulation of the vagus nerve by either pharmacological [13] or electrical methods [14,15] significantly inhibited tumor-necrosis-factor (TNF)-α release in experimental animals.

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Furthermore, cholinergic agents inhibited cytokine release from human macrophages. It has been suggested that acetylcholine released after vagus nerve stimulation significantly inhibited release of cytokines via a mechanism that required expression of the α7 subunit of the nicotinic acetylcholine receptor [15]. Therefore, when vagus nerve activity is decreased or absent, cytokines are overproduced.

Tumor necrosis factor-α is a proinflammatory cytokine involved in the pathogenesis and the progression of CHF. High circulating levels of this cytokine are found in patients with heart failure [16,17] showing a positive correlation with disease severity [2,3]. The level of TNF-α production is in part determined by promoter gene polymorphism. A polymorphism at the nucleotide position −308 G/A in the promoter region affects the expression of TNF-α gene (G = TNFA1, A = TNFA2). A TNFA2 allele is associated with high TNF-α production due to high transcriptional activation [18]. The frequency of TNFA2 allele is more prevalent in patients with rheumatoid arthritis and systemic lupus erythematosus than in healthy controls [19]. There are few reports on TNF-α polymorphism in patients with heart failure, but with inconclusive results [20–22].

The activity of autonomous nervous system (ANS), including parasympathetic function, is difficult to measure directly. A sophisticated, noninvasive and easy to perform method for assessing the activity of the ANS is analysis of heart rate variability (HRV). This simple method is useful for the evaluation of the sympatho-vagal balance at the sinoatrial level, and has large prognostic value in various cardiovascular and non-cardiovascular disorders [23,24]. A large number of studies have provided evidence for a significant correlation between lower HRV and increased mortality in patients with CHF [25–27].

The aim of this study was to investigate the connection between the autonomic imbalance and low-grade inflammatory processes in patients with CHF, by evaluating correlation between different HRV indices and serum levels of TNF-α. Furthermore, we evaluated the influence of −308 G/A TNF-α polymorphism on time domain indices of HRV and plasma concentrations of TNF-α in patients with CHF, respectively.

Materials and methods

The study protocol for this cross-sectional study was approved by the Ethics Committee of the University of Nis Faculty of Medicine, and Ethics Committee of Institute "Niska Banja", in accordance with the declaration of Helsinki. After receiving appropriate information, all recruited patients gave both their written and verbal consent for participation in the study.

Patient data

All patients had symptomatic congestive, but stable heart failure [New York Heart Association (NYHA) functional class II or III] due to left ventricular systolic dysfunction. This was confirmed by two-dimensional echocardiography performed the same day when electrocardiogram Holter had been taken off and blood samples taken for the biochemical analysis. Heart failure stage, i.e. NYHA class, was scored by the same investigator, after observation of each patient at rest, dressing, walking, and climbing up the stairs and ranged from 1 (no limitations of physical activity) to 4 (unable to carry on any physical activity) [28–30]. Ten patients (47.6%) were classified in NYHA I–II, and 11 subjects (52.4%) in NYHA III functional class.

Characteristics of the study and control subjects are presented in the Tables 1 and 2. The mean age of the two groups did not significantly differ.

Table 1
Comparison of characteristics between study and control groups.

	Study patients	Control patients	*p*
Age	64.86 ± 11.79	56.25 ± 7.19	**0.027***
Sex			
Male	14 (66.7%)	5 (62.5%)	0.833
Female	7 (33.3%)	3 (37.5%)	
TNFα (pg/mL)	5.82 ± 1.65	4.30 ± 2.72	**0.008****
EF	0.32 ± 0.09	0.65 ± 0.02	**0.000*****
EDD (mm)	62.98 ± 7.65	50.33 ± 4.03	**0.000*****
ESD (mm)	47.76 ± 10.77	30.50 ± 3.34	**0.003****
LA (mm)	44.76 ± 6.59	35.67 ± 2.34	**0.000*****
RVSP (mmHg)	33.05 ± 8.56	24.00 ± 5.29	0.092
HR holter (bpm)	69.52 ± 10.34	72.50 ± 10.90	0.500
SDNN	81.39 ± 26.25	146.21 ± 26.34	**0.000*****
DaySDNN (8 am–9 pm)	80.74 ± 29.71	119.60 ± 35.41	**0.008****
NightSDNN (11 pm–6 am)	71.96 ± 27.23	123.11 ± 29.26	**0.000*****
SDSD (ms)	31.88 ± 21.37	31.09 ± 13.98	0.924
TINN (ms)	340.30 ± 150.02	595.727 ± 172.68	**0.001*****
SDANN (ms)	68.73 ± 22.42	135.78 ± 26.38	**0.000*****
SDNNindex (bpm)	34.23 ± 15.58	44.89 ± 10.03	0.087
HRVi	10.51 ± 4.56	20.00 ± 4.20	**0.000*****
pNN50 (%)	8.54 ± 10.09	8.00 ± 4.38	0.851

* $p < 0.5$.
** $p < 0.01$.
*** $p < 0.001$.

Table 2
Study patient characteristics.

	Study patients
Beta-blocker	21 (100%)
ACE/ARB	14 (66.7%)
Digoxin	6 (28.6%)
Spironolactone	12 (57.1%)
Amiodarone	8 (38.1%)
Sodium (mmol/L)	139.80 ± 4.58
Cholesterol (mmol/L)	4.45 ± 1.65
LDL (mmol/L)	2.75 ± 1.98
HDL (mmol/L)	1.12 ± 0.18
Triglycerides (mmol/L)	1.17 ± 0.42
Creatinine (μmol/L)	99.21 ± 70.80
Urea (mmol/L)	9.11 ± 8.90
By pass	3 (14.3%)
Percutaneous coronary intervention	3 (14.3%)
Chronic stable coronary artery disease	11 (52.4%)
Previous myocardial infarction	12 (57.1%)
Diabetes mellitus	8 (38.1%)
Hypertension	14 (66.7%)
Dyslipidemia	16 (76.2%)
Smoking	6 (28.6%)
Inheritance	7 (33.3%)
Obesity	5 (23.8%)

The study group consisted of 14 (66.7%) male patients and 7 (30.4%) female patients. Three women (37.5%) were assessed in the control group. All patients were stable with regard to symptoms and therapy for at least 1 month before the measurements conducted within this study, and in sinus rhythm. Exclusion criteria were active infection, allergy, inflammatory diseases, cancer, and treatment with anti-inflammatory drugs.

The patients were on standard medication consisting of angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (66.7%), diuretics (71.4%), spironolactone (57.1%), digoxin (28.6%), beta-blockers (100.0%), low-dose aspirin (81.0%), statin (76.2%), and amiodarone (38.1%). History of diabetes mellitus was also assessed. Eight CHF patients (38.1%) had diabetes, and none in the control group. The control group of patients consisted of healthy subjects who did not use any medication prior to or during the examination that might affect autonomic nervous activity.

Smoking status was categorized as non-smoker or former smoker, and current smoker.

Heart rate variability analysis

An ambulatory two-channel 24-hour electrocardiogram was recorded from the patients during their everyday activities, using the Del Mar Avionics models 5268-505 MPA/RACQ RACQ: 2.15 (Irvine, CA, USA), and analyzed according to the recommendations of the Task Force of the European Society of Cardiology (ESC) and the North American Society of Pacing (NASPE) [24].

Time-domain analysis refers to statistics that are derived directly from the measurement of the normal-to-normal (N-N) intervals (i.e. intervals between consecutive QRS complexes resulting from sinoatrial discharge) and to statistics calculated from the differences between successive N-N intervals [23,24,28]. The following time domain variables were computed for each subject: standard deviation of the normal-to-normal QRS intervals (SDNN), the standard deviation of mean R-R intervals in 5-minute recordings (SDANN), and proportion derived by dividing the number of interval differences of successive N-N intervals greater than 50 ms by the total number of N-N intervals (pNN50). DaySDNN was measured during the day from 8 am until 9 pm, while NightSDNN was evaluated in the night period from 11 pm to 6 am. HRV triangular index (HRVi), a geometric measure of HRV developed by Malik and coworker [29] preferably computed on long term-recordings, is an index of total variability such as SDNN and SDANN [24]. Triangular interpolation of NN intervals (TINN) is another geometric time domain measure.

Blood sampling and laboratory analyses

Blood samples were collected from each subject between 9 am and 10 am after a fasting period of at least 10 h. Concentration of TNF-α was measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications (Biolegend, Fell, Germany). The limit of detection for TNF levels was 2 pg/ml. All samples were measured in duplicates.

All of the assays were performed in the laboratory for functional genomics and proteomics, Research Center for Biomedicine, and technicians were blinded to the clinical characteristics and outcomes of the patients.

Identification of the TNF-α gene polymorphism and genotyping

Genomic DNA was isolated from the whole blood taken on ethylenediaminetetraacetic acid and from peripheral blood smear using conventional methods of sodium dodecyl sulfate lyses, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation and also using Quiagen DNA Isolation Kit (Quiagen GmbH, Hilden, Germany). Bi-allelic polymorphism (G/A substitution) within the TNF-α gene was determined by polymerase chain reaction – restriction fragment long polymorphism technique. The regions containing the *TNF-α-308* locus were amplified using the primers *TNF-α-308-1* (5′-AGGCAATAGGTTTTGAGGGCCAT-3′) and *TNF-α-308-2* (5′-ACACTCCCCATCCTCCCTGCT-3′). A 117 base pair (bp) polymerase chain reaction (PCR) product was generated using the following reaction conditions: initiation at 95 °C for 15′; 35 cycles of denaturation at 94 °C for 1′; annealing at 50 °C for 1′ and extension temperature 72 °C for 1′; ending with 4 °C. PCR products were analyzed electrophoretically in 2% agarose gel and visualized under ultraviolet light. The amplified DNA was incubated with *NcoI* and the treated fragments were analyzed by 8% polyacrylamide gel and visualized by way of silver staining. Interpretation was as follows: a single band at 117 bp identified individuals homozygous for adenine at TNF-α 7308 locus; two bands at 97 and 20 bp identified individuals homozygous for guanine; three bands at 117, 97 and 20 bp identified individuals heterozygous at the *TNF-α-308* locus.

Statistical analysis

The data analysis was performed by Statistical Package for Social Sciences (SPSS 16.0; Chicago, IL, USA). Baseline characteristics are presented as frequencies or means with SDs. Student's *t*-test and ANOVA were used for comparing the groups' characteristics with normal distribution. Nonparametric data were analyzed using Chi-square test or two-tailed Fisher's exact test. The association between TNF-α levels and HRV parameters was evaluated by Pearson correlation analysis. A *p*-value less than 0.05 was considered to be a measure of statistical significance for all statistical tests used.

Results

Correlation between HRV parameters and TNF-α levels

The first goal of this study was to find a correlation between TNF-α level and both echocardiography and HRV parameters. The Pearson's correlation coefficients with significance levels for the correlations between various HRV measures and TNF-α levels are shown in Table 3. TNF-α levels were significantly and inversely correlated with a time-domain parameter of long-term variability SDNN ($r = -0.542$, $p < 0.05$). A similar result was found for HRVi, a geometric measure of HRV ($r = -0.556$; $p < 0.05$). However, the correlation between TNF-α levels and heart rate (HR) was not significant ($p = 0.828$). Stronger correlations were found in female patients (SDNN: $r = -0.940$, $p < 0.01$; SDANN: $r = -0.826$, $p < 0.05$) and patients with diabetes mellitus (DM) (SDNN: $r = -0.961$, $p < 0.001$). The pNN50, an indirect marker of cardiac vagal activity, was not significantly correlated with plasma concentration of TNF-α.

The mean plasma TNF-α level was 5.82 ± 1.65 pg/mL. We classified patients into a high TNF-α group (≥5.50 pg/mL) and a low TNF-α group (<5.50 pg/mL) and compared variables in these groups (Table 4). There were no significant differences in age, mean arterial BP, heart rate (HR), sodium levels, and creatinine levels among the two groups of patients. When comparing HRV parameters across the two TNF-α groups, significantly lower values for nearly all HRV variables (SDNN, Night SDNN, SDANN, TINN, and HRVi), were associated with lower TNF-α level. Two subgroups of patients, classified according to the plasma TNF-α level, did not differ significantly in NYHA functional class, and furthermore, the use of β-blockers, angiotensin-converting enzyme inhibitors, digitalis (digoxin), spironolactone, and amiodarone was similar in both groups. As shown, higher TNF-α was associated with diabetes mellitus.

Table 3
Correlations between TNFα level and HRV parameters.

	SDNN	DaySDNN (8 am–9 pm)	NightSDNN (11 pm–6 am)	SDANN (ms)	SDNNindex (bpm)	HRVi	pNN50 (%)	SDSD (ms)	TINN (ms)
r	−0.542	−0.444	−0.529	−0.426	−0.365	−0.556	−0.177	−0.079	−0.510
p	**0.011***	0.065	**0.024***	0.069	0.113	**0.011***	0.483	0.756	**0.031***

* $p < 0.05$.

Table 4
Differences in patient characteristics between patients with low and high TNFα plasma levels (below and above mean TNFα).

Number of patients (%)	Low TNFα 12 (57.1%)	High TNFα 9 (42.9%)	*p*
Age	62.67 ± 11.87	67.78 ± 11.70	0.338
Systolic BP (mmHg)	134.83 ± 22.67	130.00 ± 14.14	0.565
Diastolic BP (mmHg)	78.75 ± 11.10	84.38 ± 10.50	0.272
EF	0.31 ± 0.10	0.34 ± 0.07	0.465
EDD (mm)	65.00 ± 9.17	60.28 ± 4.07	0.132
ESD (mm)	50.67 ± 13.23	43.89 ± 4.40	0.120
LA (mm)	45.25 ± 8.20	44.11 ± 3.89	0.679
RVSP (mmHg)	31.67 ± 6.60	34.89 ± 10.81	0.407
HR holter (bpm)	69.25 ± 12.08	69.89 ± 8.13	0.893
Sodium (mmol/L)	140.09 ± 3.73	139.44 ± 5.68	0.763
SDNN	91.52 ± 25.92	67.88 ± 20.97	**0.032***
DaySDNN (8 am–9 pm)	88.93 ± 33.78	70.51 ± 21.49	0.200
NightSDNN	82.87 ± 31.35	58.32 ± 12.44	**0.042***
SDSD (ms)	29.29 ± 20.51	35.12 ± 23.37	0.581
TINN (ms)	406.27 ± 158.49	257.84 ± 91.49	**0.028***
SDANN (ms)	76.74 ± 21.37	57.71 ± 20.00	**0.032***
SDNNindex (bpm)	36.74 ± 16.93	31.17 ± 14.11	0.441
HRVi	12.49 ± 4.65	8.09 ± 3.22	**0.027***
pNN50 (%)	7.48 ± 6.49	9.60 ± 13.11	0.671
Cholesterol (mmol/L)	5.10 ± 1.84	3.51 ± 0.67	**0.047***
LDL (mmol/L)	3.53 ± 2.45	1.78 ± 0.45	0.207
HDL (mmol/L)	1.19 ± 0.20	1.02 ± 0.09	0.113
Triglycerides (mmol/L)	1.25 ± 0.50	1.06 ± 0.26	0.371
Creatinine (μmol/L)	77.42 ± 17.78	126.45 ± 101.19	0.216
Urea (mmol/L)	6.10 ± 1.63	12.86 ± 12.65	0.176
ACE/ARB	7 (58.3%)	7 (77.8%)	0.510
Digoxin	5 (41.7%)	1 (11.1%)	0.125
Spironolactone	7 (58.3%)	5 (55.6%)	0.899
Amiodarone	5 (41.7%)	3 (33.3%)	0.697
NYHA			
I–II	5 (41.7%)	5 (55.6%)	0.528
III	7 (58.3%)	4 (44.4%)	
TNF308			
GG	6 (50.0%)	1 (11.1%)	0.061
GA	6 (50.0%)	8 (88.9%)	
By pass	1 (8.3%)	2 (22.2%)	0.368
Percutaneous coronary intervention	2 (16.7%)	1 (11.1%)	0.719
Chronic stable coronary artery disease	5 (41.7%)	6 (66.7%)	0.256
Previous myocardial infarction	7 (58.3%)	5 (55.6%)	0.899
Diabetes mellitus	2 (16.7%)	6 (66.7%)	**0.020***
Hypertension	6 (50.0%)	8 (88.9%)	0.095
Dyslipidemia	10 (83.3%)	6 (66.7%)	0.375
Smoking	5 (41.7%)	1 (22.2%)	0.125
Inheritance	3 (25.0%)	4 (44.4%)	0.350
Obesity	3 (25.0%)	2 (22.2%)	0.882

* $p < 0.05$.

Distribution of TNF-α alleles and genotypes

The prevalence of the TNF-α gene promoter polymorphism G/A at position −308 seen in our study, is similar to the ones reported by other investigators [31,32] (Table 5). The observed genotype distribution in HF patients did not show differences in comparison to the control group of healthy unrelated, randomly selected normal individuals: TNFA1/TNFA2 = 0.67/0.33 (CHF) or 0.62/0.38 (control) as already published [20,33].

Plasma levels of TNF-α were elevated in patients with CHF compared with the control subjects (5.82 ± 1.65 pg/ml vs. 4.30 ± 2.72 pg/ml, $p < 0.01$). Our findings correspond to the results of other studies [20,34,35]. After adding TNF-α 308 genotype as a discriminator, we have found that higher plasma levels were associated with TNFA2 genotype (TNFA1 vs. TNFA2) both in study patients (4.62 ± 0.58 pg/ml vs. 6.42 ± 1.70 pg/ml, $p < 0.01$) and the control group (2.05 ± 1.35 pg/ml vs. 5.66 ± 2.43 pg/ml, $p < 0.05$).

The HRV indices differed significantly between TNFA1 and TNFA2 carriers (Fig. 1). In line with our previous findings that TNF-α was inversely correlated with HRV measurements, and that TNF-α was lower in patients with TNFA1 genotype, we have found that the TNF-α gene promoter polymorphism G/A at position −308 carriers had lower SDNN ($p < 0.01$), Night SDNN ($p < 0.01$), and SDNN index ($p < 0.05$).

Discussion

In the present study, we confirmed earlier observations, which suggest an inverse correlation of TNF-α levels with HRV indices in patients with CHF. In our study, SDNN, SDANN, and HRVi were significantly and inversely correlated with plasma levels of TNF-α, indicating that higher serum TNF-α levels were associated with lower HRV. Stronger correlations were found in female patients, patients with diabetes mellitus (DM) and TNFA2 allele (an "A" at position −308A).

Our results are in accordance with those of Malave et al. [34] who reported an inverse relationship between increased levels of circulating TNF-α and indices of decreased HRV in patients with mild-to-moderate heart failure. These authors showed that circulating levels of TNF-α are a stronger independent predictor of depressed HRV than the circulating level of norepinephrine, which

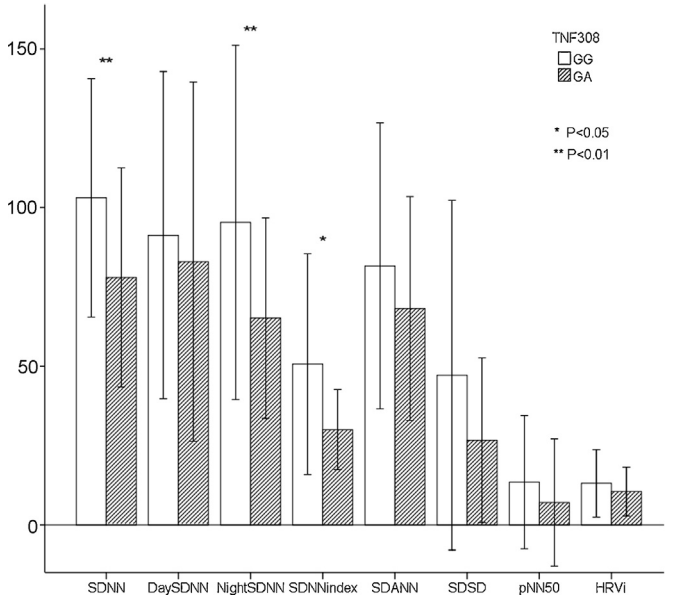

Fig. 1. Heart rate variability parameters in patients with different tumor necrosis factor (TNF) 308 genotypes.

Table 5
Distribution of TNF-α genotypes and alleles.

	Genotype frequency			Allele frequency	
	TNF308 G/G	TNF308 G/A	TNF308 A/A	TNF308 G	TNF308 A
CHF frequency (number of patients)	0.67 (14)	0.33 (7)	0.00 (0)	0.83	0.17
Control frequency (number of patients)	0.62 (5)	0.38 (3)	0.00 (0)	0.81	0.19
	$\chi^2 = 0.045$, $p = 0.833$			$\chi^2 = 0.035$, $p = 0.851$	

is encouraging for the possible existence of a direct relationship. Straburzyńska-Migaj et al. [36] reported the significant negative correlations between time-domain HRV parameters and TNF-α soluble receptors (sTNF-RI, sTNF-RII) and IL-6 levels, respectively.

These findings are in contrast to a study in patients with decompensated heart failure: there was strong evidence of an inverse relation between HRV and IL-6 levels in systemic circulation whilst TNF-α levels did not correlate with any of the HRV measures [37].

CHF is characterized by an ongoing inflammatory response showing a positive correlation of inflammatory cytokines, such as TNF-α, IL-1b, IL-6, IL-18, with disease severity and prognosis [1–3]. Mechanisms of immune activation in CHF are still indeterminate. Autonomic imbalance is closely related with them and, according to our findings, might be partly also due to the immune activation.

TNF-α is a potent proinflammatory cytokine released by most cell types with pleiotropic effects. Since Levine and coworkers [17] reported that the levels of TNF-α were elevated in the settings of CHF, several studies have shown a correlation between plasma TNF-α levels and the progression of heart failure [16,38], due to cardiac myocyte hypertrophy [39], contractile dysfunction [40], and left ventricular remodeling [3]. However, rapid TNF-α biosynthesis by cardiac myocytes and nonmyocardial cell types within the myocardium, and some of its protective effects in acute ischemic injury, suggest that TNF-α has a complex, yet not completely known role in heart diseases [41].

Some studies have provided evidence that cardiac production is not the main source of raised peripheral cytokine plasma concentrations, especially in patients with mild and moderate CHF [42,43], and that the bioactivity of TNF-α in the serum could be modulated by sTNF-Rs [44–46]. This suggests that induction of cytokines in the myocardium is a relatively late event in the pathogenesis of CHF and partly explains contradictory results of human studies. However, plasma levels of TNF-α correlates with mRNA expression in the myocardium and thus may serve as an appropriate marker of myocardial cytokine activation in spite of influence by other factors, such as clearance and production of TNF-α by nonmyocardial tissues [42].

HRV has demonstrated decreased parasympathetic and increased sympathetic reactivity [47] and is associated with elevated inflammatory markers in patients with diabetes mellitus [48,49] or impaired glucose tolerance [50], hypertension [51,52], mental depression [53], and coronary artery disease [54]. In the Framingham study, SDNN was a significant predictor of all-cause mortality after adjusting for other risk factors [55]. SDNN and SDANN reflect both sympathetic and parasympathetic modulation of HR and reduced SDANN and SDNN values usually indicate relative sympathetic dominance [56].

The autonomic imbalance in heart failure, especially changes in vagus nerve control of HR, become apparent at a very early developmental stage of left ventricular dysfunction [57], and it could be an interesting target for new drugs in patients with CHF. After the evidence from experimental heart failure model on efficacy of electrical vagus nerve stimulation in CHF [58], the first-in-human study performed on 32 patients with CHF showed significant improvements in NYHA class, quality of life, 6-min walk test, left ventricular ejection fraction, and left ventricular systolic volumes [59,60]. Randomized controlled clinical trials of adequate size are needed for confirming vagal nerve stimulation as a new therapeutic strategy for CHF. Our findings suggest that chronic low-grade systemic inflammation which accompanies autonomic imbalance in CHF could be a useful marker for noninvasive selection of patients who could benefit from vagal nerve stimulation or immunological-mediated therapies such as non-specific immunomodulation therapy or intravenous immunoglobulins [61].

It should be noted that there are important limitations to interpretation of the present findings. First, the number of control patients was relatively small in size in comparison with study patients. But, even though, we are confident that restrictive inclusion criteria, determined homoscedasticity of variance prior analysis, and homogeneity of obtained results for control patients can be enough for valid statistical comparison with study patients. In addition, some similar designed experiments [34] in their analyses used quite similar sample sizes. Another limitation of the study is that only one cytokine was analyzed. Although there is considerable interest in examining different proinflammatory cytokines in CHF patients as they are biomarkers of disease severity and prognosis, TNF-α is considered to be one of the main predictors of worsening heart failure and increasing mortality. In a recently published review [62], the authors summarized a large number of significant studies where the role of various inflammatory biomarkers in heart failure was studied. In the vast majority of them, TNF-α was posited as one of the most specific cytokines related to heart failure, since its plasma elevation significantly correlates with both disease progression and prediction. In line with this fact is the evidence that administration of TNF-α to experimental animals or its transgenic over expression can replicate the heart failure phenotype [63,64], which further supports the importance of the role of TNF-α in the disease. Taking all this into consideration, we have chosen this cytokine to analyze and to evaluate its relation with HRV. However, our study does not consider other cytokines to be less significant nor preclude their role in pathophysiology of heart failure.

The present study reports novel findings regarding the influence of polymorphisms −308 A/G. Even though we have found significant correlation of both autonomic imbalance assessed by HRV and TNF-α level with polymorphisms −308 A/G, these results also should be taken with reserve considering the relatively small sample size.

In conclusion, the results of the present study indicate that an increased serum TNF-α is significantly associated with reduced HRV indices, suggesting that activation of the immune system in patients with CHF is closely related to autonomic imbalance.

Conflict of interest

The authors state no conflict of interest.

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