

## RESEARCH ARTICLE

# Relationship of Endothelin-1, Tumor Necrosis Factor- $\alpha$ and Interleukin-6 with the Progression of Heart Failure

Mona Yolanda<sup>1,4</sup>, Marsetio Donoseputro<sup>2</sup>, and Anwar Santoso<sup>3</sup>

**BACKGROUND:** Heart failure, a new epidemic of cardiovascular disease, has become an important issue and its prognosis is poor. Heart failure is the condition where the impaired heart cannot pump enough blood to provide the needs of metabolic tissues and organs. Early diagnosis of heart failure is really crucial to determine the success of treatment and to prevent further myocardial dysfunction and worsening clinical symptoms. This condition can be worsened by ET-1, which triggers the secretion of IL-6 and TNF- $\alpha$  as pro inflammation factors. In Systolic Heart Failure, systolic function changes are accompanied by changes in diastolic function. Thus, the condition of systolic heart failure is worse than Diastolic Heart Failure. The purpose of this study is to assess the relationship of ET-1 with diastolic and systolic heart failure groups and the relationship of ET-1 with TNF- $\alpha$  and IL-6 as pro inflammation factors.

**METHODS:** The design of this study was cross-sectional analysis on 62 patients with heart failure, grouped according to the classification of diastolic and systolic heart failure.

**RESULTS:** A significant positive correlation of ET-1 with diastolic and systolic heart failure was found ( $p = 0.008$ ;  $r = 0.324$ ). A significant positive correlation was also found between ET-1 and IL-6 ( $p = 0.001$ ;  $r = 0.393$ ), but a less significant correlation was noted between ET-1 and TNF- $\alpha$  ( $p = 0.201$ ;  $r = -0.158$ ).

**CONCLUSIONS:** ET-1 has the strongest correlation ( $p=0.033$ ) with prevalence ratio 3.930 and can differentiate between Diastolic and Systolic Heart Failure.

**KEYWORDS:** Endothelin-1 (ET-1), Interleukin-6 (IL-6), Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), heart failure.

## Introduction

Heart failure has become such a highly important public health problem that it is named “a new epidemic of cardiovascular disease”, with a poor prognosis. More than 4 million new cases of heart failure are diagnosed each year in the United State. Worldwide, it is estimated that there are 15 million new cases of heart failure in a year. The number may increase rapidly in association with the increased number of aging populations.<sup>1</sup>

The diagnosis of heart failure can be properly made based on clinical presentations, history, physical activity tolerance, and several other relevant investigations. It is easy to identify the level of heart failure, ranging from moderate to severe degree, mainly based on the signs and symptoms. The problem appears when defining and diagnosing heart failure at the initial stage where no apparent signs and symptoms present.<sup>2</sup>

In this study, we tried using plasma ET-1, IL-6 and TNF- $\alpha$  for early diagnosis of heart failure and using BNP and echocardiography for classifying the two groups of heart failure i.e. diastolic and systolic heart failure. In Systolic Heart Failure, systolic function changes are accompanied by changes in diastolic function. Thus, the condition of systolic heart failure is worse than Diastolic Heart Failure.

<sup>1</sup>Prodia Clinical Laboratory, Jakarta

<sup>2</sup>Faculty of Medicine Airlangga University, Surabaya

<sup>3</sup>Faculty of Medicine Udayana University, Bali

<sup>4</sup>Post Graduate Program, Hasanuddin University, Makasar

ET-1 is a strong vasoconstrictor and it can induce proliferation of VSMC and production of fibroblasts, modulate synthesis extracellular matrix that causes hypertrophy of VSMC, and it can affect vascular permeability and inflammation.<sup>3</sup> Exercise causes

up-regulation of expression of myocardial ET-1; it is stated that ET-1 plays a role in maintaining the heart function. Hypoxia is a strong stimulus to the synthesis of ET-1, which is highly important in ischemia.<sup>4</sup>

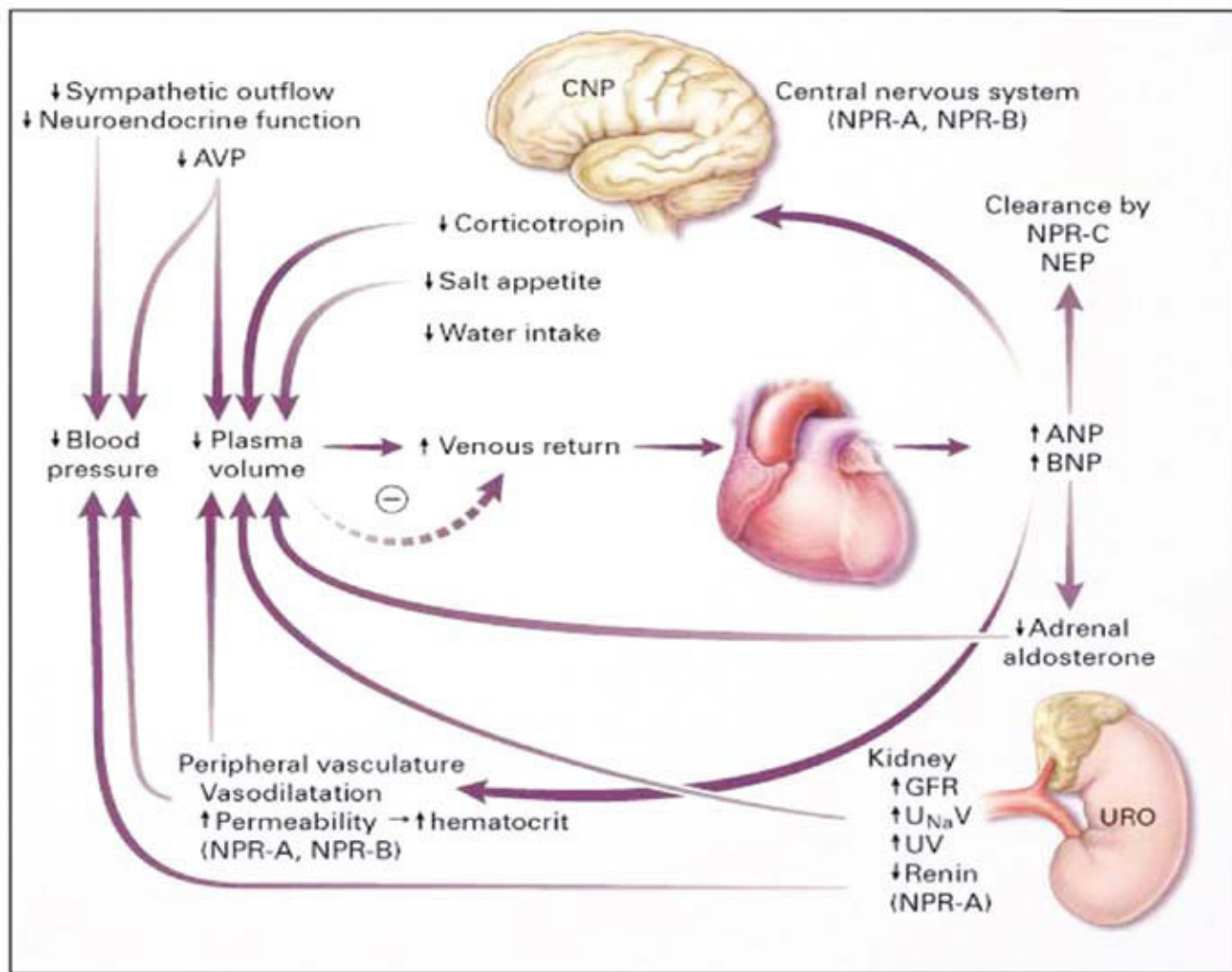


Figure 1. Physiologic effect of Natriuretic Peptide is induced by the heart when venous return increases<sup>9</sup>  
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The level of ET-1 is positively correlated with the progression of heart failure, increased mortality rate, and clinical symptoms in patients with heart failure; and it can also predict the resilience congestive life 4, 5, 6. ET-1 also induces production of cytokines in human VSM. ET-1 induces L-6 from the SMC. IL-6 is a multifunctional cytokines that causes stimulus of acute phase reactions such as C-reactive protein (CRP).

Natriuretic peptide works as a vasodilator that has an hypotensive effect, causes natriuresis and diuresis, inhibits activity of the sympathetic nervous system and several hormonal systems, including the system of RAAS endothelin-1, cytokines, and vasopressin; and it can prevent pathophysiologic mechanisms responsible for ventricular and vascular hypertrophy and remodeling.<sup>7</sup>

TNF- $\alpha$  causes structural changes such as heart muscle apoptosis and hypertrophy resulting in dilated cardiomyopathy. Increased TNF- $\alpha$  causes left ventricle dysfunction and collagen remodeling by changes in the heart muscles. IL-6 is a multifunctional cytokine that mediates both immunological response and inflammation that cause myocardial injury <sup>9</sup>. IL-6 and TNF- $\alpha$  are associated with the degree of illness and degree of LVH activation on sympathetic system and rennin-angiotensin. <sup>10</sup> Thus TNF- $\alpha$  and IL-6 are not directly involved in the occurrence of hypertrophy that will cause the heart failure.

## Methods

The design of this study was cross-sectional analysis on 62 patients with heart failure, grouped into systolic (Ejection Fraction<40%) and diastolic heart failure (Ejection Fraction>40%) as assessed by echocardiographic measurement. The diagnosis of heart failure was made based on the Framingham criteria. Changes in ET-1, IL-6, TNF- $\alpha$ , and BNP were determined. None of the patients had any signs of acute or chronic disease.

### ASSAYS OF BIOCHEMICAL MARKERS

Plasma levels of ET-1, IL-6, and TNF- $\alpha$  were measured by ELISA methods using R&D Systems, Inc, Minneapolis, USA. Plasma levels of BNP were determined by fluorescence immunoassay method using Triage® BNP test (Biosite Inc, San Diego, California).

### STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS for Windows version 13.0 software. P value of less than 0.05 was used to indicate statistical significance. General description of data was made by univariate analysis to assess mean, median, and standard deviation. For comparing all factors between groups of patients of systolic and diastolic heart failure we used T-test. Multiple regression test was used to analyze correlation of different factors.

## Results

Statistical analysis made on the two groups of heart failure classified as Systolic Heart Failure (SHF) and Diastolic Heart Failure (DHF) used independent samples t-test to see differences in the factors that contributed to the degree of heart failure namely the levels of ET-1, IL-6, and TNF- $\alpha$ .

**Table 1. Clinical characteristics and biochemical parameters of 62 patients with heart failure**

Parameter	Mean $\pm$ SD
Age (yr)	57 $\pm$ 10.75
ET-1 (pg/ mL)	1.56 $\pm$ 1.17
TNF- $\alpha$ (pg/ mL)	9.03 $\pm$ 12.95
IL-6 (pg/ mL)	10.81 $\pm$ 24.77
BNP (pg/ mL)	524.44 $\pm$ 556.67

BNP: Brain Natriuretic Peptide  
ET-1: Endothelin-1  
TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$   
IL-6: Interleukin-6

**Table 2. Clinical characteristics and biochemical parameters based on DHF and SHF of 62 patients with heart failure**

Parameter	Mean $\pm$ SD		p
	DHF	SHF	
Age (yr)	59.11 $\pm$ 11.92	56.54 $\pm$ 9.95	0.342
ET-1 (pg/mL)	1.17 $\pm$ 0.64	1.81 $\pm$ 1.36	0.008
TNF- $\alpha$ (pg/mL)	6.57 $\pm$ 5.74	10.60 $\pm$ 15.81	0.497
IL-6 (pg/mL)	6.57 $\pm$ 11.47	13.49 $\pm$ 30.19	0.030
BNP (pg/mL)	288.84 $\pm$ 422.77	673.84 $\pm$ 583.65	0.000
Ejection Fraction	57.94 $\pm$ 15.77	26.61 $\pm$ 9.70	0.000

BMI= Body Mass Index; HDL= High Density Lipoprotein; LCAT=Lecithin Cholesterol Acyl Transferase; CETP=Cholesterol Ester Transfer Protein; sdLDL=small dense Low Density Lipoprotein; AIP=Atherogenic Index of Plasma; p=probability, \* = statistically significance ( $p < 0,05$ )

**Table 3. Multiple logistic regression analysis of 62 patients with heart failure**

Parameter	p	Exp (B)	95% CI
ET-1	0.033	3.930	1.119 - 13.803
BNP	0.104	2.787	0.809 - 9.600

BNP: Brain Natriuretic Peptide  
ET-1: Endothelin-1

Based on the value of EF, The Subjects could be grouped into diastolic ( $EF > 40\%$ ) and systolic heart failure ( $EF < 40\%$ ). BNP concentrations relate inversely with EF values. The greater the concentration of BNP, the smaller the EF values will be. This shows that the situation is getting worse. Multiple regression analysis showed that ET-1 has the stronger correlation ( $p=0.033$ ) compared with BNP, with prevalence ratio of 3.930 and thus could differentiate DHF and SHF.

## Discussion

The pathophysiologic hallmark of heart failure is the occurrence of remodeling pathology in tissue of the heart that is triggered by a change in cardiac load after occurrence of myocardial injury due to activation and modulation for a long period of various neurohormonal systems and autocrine/paracrine, among others adrenergic nervous system, rennin-angiotensin-aldosterone system, arginine-vasopresin, endothelin, inflammation cytokines, and growth factors 11.

In this study we tried to prove that ET-1 and inflammatory factors (TNF- $\alpha$  and IL-6) were involved in the process of heart failure in influencing the degree of the heart failure. The results of the research showed that there was significant positive correlation of ejection fraction with BNP in the groups of heart failure of Systolic and Diastolic Heart Failure, there was weak positive correlation among ET-1, IL-6 and

TNF- $\alpha$  in heart failure patients, and there was strongest correlation ( $p=0.033$ ) of ET-1 with prevalence ratio 3.930 in the groups of Systolic and Diastolic Heart Failure.

Some hormones and neurotransmitters such as endothelin, arginine-vasopresin, and catecholamines directly stimulate the natriuretic peptide hormone. Increased pressure on the ventricle wall, reflecting increased intravascular volume, is the dominant stimulus of this natriuretic peptide.

ET-1 is a strong vasoconstrictor and stimulator of cell proliferation in a network within and outside the cardiovascular system ( $p=0.033$ ) ( $p=0.033$ ). 1,4 At the time of vasoconstriction, the body adaptive system has to overcome the secreted natriuretic peptide BNP in the case of heart failure. BNP is secreted by the heart tissue as a normal process of the cardiovascular system

to maintain homeostasis.<sup>8</sup> BNP works as a vasodilator having hypotensive effect, causing diuresis and natriuresis, and prevents sympathetic nervous system activity and several hormonal systems, including RAAS system, ET-1, cytokines, and vasopressin.<sup>7</sup> According to Marguiles K et al; Wei C et al; and Schirger et al there is increased expression of ET-1 in heart failure that can increase the degree of heart failure through progression of hemodynamic effects such as remodeling of heart muscles vascularities.<sup>12</sup>

In addition, results of this study also showed that the concentration of BNP in the SHF was higher than DHF ( $p = 0,000 *$ ). This was due to the chemical-physical features of BNP as a biochemical receptor secreted in certain circumstances, in this case the volume overload of SHF that was more severe than DHF, so the value of BNP was greater concurrently with the progression of the heart failure.

ET-1 induces production of cytokines in human VSMC. ET-1 also induces production IL-6 from the SMC. Ability of ET-1 to initiate inflammatory response on VSMC activation was enhanced by the human transcript proinflammatory NF- $\kappa$ B and stimulation of production of cytokines. Browatzki et al found that ET-1 induced IL-6 activation through NF- $\kappa$ B in human vascular smooth muscles and stimulation of production of other cytokines.

Other studies point out that IL-6 from stimulated ET-1 that is mediated through ET-A and ET B-receptors reduces the production of IL-6. <sup>5,13</sup> PRAISE (Prospective Randomized Amlodipine Survival Evaluation) multicentre study has proven that the decrease in the mortality of CHF is associated with the decrease in plasma concentration of IL-6. <sup>9</sup>

TNF- $\alpha$  has a higher concentration in patients who have prolonged heart failure than new heart failure patients. IL-6 first increases rather than TNF- $\alpha$

in the pathogenesis of heart failure, both involved in determining the degree of heart failure. <sup>14</sup> TNF- $\alpha$  causes structural changes in the myocardium such as apoptosis and hypertrophy resulting in dilated cardiomyopathy. Recent studies point out declined contractibility of the myocardium, arterial stiffness, and coronary action is triggered through ET-1. <sup>9</sup> TNF- $\alpha$  is a key mediator in the immune and inflammatory responses.

In our present study, there was a weak positive correlation found between ET-1, IL-6 and TNF- $\alpha$  in heart failure patients. IL-6 and TNF- $\alpha$  were secreted from tissue cardiomyosites and entered blood circulation. In another previous study, IL-6 and TNF- $\alpha$  showed to have strong correlation with the progression of heart failure. This finding was due to the different type of samples used in the study as compared with the ones used in our study. In our study, we used samples obtained from plasma and in the above other previous study the samples were taken from tissue cardiomyosites. Therefore, IL-6 and TNF- $\alpha$  levels from the tissues were higher than those in the plasma, hence the weak positive correlation found in our study between ET-1, IL-6 and TNF- $\alpha$ .

ET-1 has the most significant role in determining the degree of illness due to the heart's being unable to distinguish SHF from DHF ( $p = 0,033$ ), with a prevalence rate of 3.930, which means that individuals with heart failure having a concentration of ET-1  $>1:27$  pg / mL get an incidence rate of SHF 3.93 times greater than that of DHF.

In conclusion, ET-1 is a factor that can trigger vasoconstriction, stimulate VSMC secreted by IL-6 and TNF- $\alpha$  as inflammatory factors, and it is most significantly associated with Diastolic and Systolic Heart Failure.

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