RESEARCH ARTICLE

Association among Soluble Tumor Necrosis Factor Receptor I, Matrix Metalloproteinase-9, Procollagen C-terminal Propeptide type I, and N-Terminal proBrain Natriuretic Peptide in Heart Failure

Mona Yolanda[1,2*], Marsetio Donosepoetro[3], Anwar Santoso[4], Mansyur Arief[1]

[1]Post Graduate Program in Clinical Biochemistry, Hassanudin University, Jl. Perintis Kemerdekaan Kav. 10, Makassar, Indonesia
[2]Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung 40114, Indonesia
[3]Faculty of Medicine, Airlangga University, Jl. Mayjen Prof. Dr. Moestopo No. 47, Surabaya, Indonesia
[4]National Cardiovascular Center-Harapan Kita, Jl. Letjen. S. Parman Kav 87, Slipi, Jakarta, Indonesia
*Correspondence: mona.yolanda@prodia.co.id

Abstract

BACKGROUND: Heart failure (HF) is associated with an increased expression of proinflammatory cytokines, especially soluble tumor necrosis factor receptor I (sTNFR I), but the underlying mechanism to the relationship between sTNFR I activation and the progression of HF is not yet fully understood. This study aims to see the association between sTNFR I, MMP-9, PICP, and NT-proBNP in the progression of HF.

METHODS: This was a cross sectional study which recruited 45 subjects with HF confirmed by echocardiography and NT-proBNP. Concentration sTNFR I, MMP-9, and PICP were measured using ELISA method, whereas NT-proBNP concentration was measured using ECLIA method. Univariate linear regression analysis, path analysis and General Linear Model were used to determine which parameters played the most significant role in HF.

RESULTS: Results of the univariate linear regression and path analysis showed there was a linear relationship between sTNFR I with MMP-9, with R square of 25.8% (p = 0.00; r = 0.508), R square sTNFRI and MMP-9 with PICP was 14.4% (p = 0.038; r = 0.379) and R square MMP-9 and PICP with NT-proBNP was 39.6% (p = 0.00; r = 0.629). From the General Linear Model we found that the important predictor for HF was through MMP-9 and PICP.

CONCLUSION: sTNFR I as a proinflammatory factor is one of the factors involved in the heart failure as seen by NT-proBNP through activation of fibrosis (PICP) and remodeling factor (MMP-9).

KEYWORDS: sTNFR I, MMP-9, PICP, NT-proBNP, heart failure.

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Introduction

Heart failure is seen as a complex situation due to changes in the structure and function of the myocardium as well as activation of several neurohormonal systems that have an effect on this process. Neurohormonal activation complex aims to improve the mechanical condition of the cardiac hormone, such as circulation and local pro-inflammatory cytokine that plays an important role in disease progression (1).

Heart failure is associated with an increased expression of proinflammatory cytokines, especially TNF-α (tumor necrosis factor-α), but the underlying mechanism of the relationship between TNF-α activation and the progression of the heart failure is not yet fully understood. Previous studies consistently showed a direct relationship between concentrations of cytokines and worsening of the heart

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failure (2). In this study we used sTNFR I, which is a TNF-α receptor, as a marker for pro-inflammatory cytokines.

The matrix metalloproteinases (MMPs) are proteinases that play a role in the degradation of extracellular matrix. This study using MMP-9 had a significant contribution to explain the role in cardiovascular disease processes involved in vascular remodeling, atherosclerotic plaque instability, and left ventricular remodeling after myocardial infarction (3).

Fibrosis contributes to rigidity. Myocardial fibrosis would disrupt the coordination of excitation-contraction in both diastolic and systolic. In this study biochemical marker PICP (C-terminal collagen type I Propetide) was used as a biochemical marker for collagen synthesis (4).

The American College of Cardiology Foundation/ American Heart Association Practice Guidelines : Focus Update 2009 described the determination of heart failure according to New York Heart Association (NYHA, and examination of the role of B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP), a comprehensive evaluation on heart failure patients. This guide explained that the examination of NT-proBNP and BNP is recommended for clinical determination of patients who showed symptoms of heart disease. This guide explains that the examination of NT-proBNP and BNP is recommended for clinical determination of patients who showed symptoms of heart disease. Measurement of natriuretic peptides (BNP and NT-proBNP) is useful in the evaluation of patients in emergency situations with a clinical diagnosis of heart failure being unclear for risk stratification by the level of evidence A class (5).

The study was conducted to see the role of pro-inflammatory cytokine that induces fibrosis and remodelling factors in heart failure.

Methods

Forty five patients with heart failure, aged above 40 years were enrolled in this cross sectional study. Participants signed informed consents to have personal data such as height, weight, serum measurements, blood pressure, and waist circumference. The study's proposal was approved by The Health Research Ethics Committee of The Faculty of Medicine Hasanuddin University of Makassar and Udayana University of Denpasar.

sTNFR I (cat#SRT100) and MMP-9 (cat#DMP900) were measured by ELISA method, Enzyme Linked Immunosorbent Assay kit (R&D Systems), PICP (cat#8003) was measured by Enzyme Immunoassay kit (Quidel^R), and NT-proBNP (cat#04842464) was measured by electrochemiluminescence immunoassay (Roche). Sera were separated from whole blood specimens after centrifugation, and immediately kept at −20ºC until assayed. All assays were performed according to manufacturers' instructions.

Statistical analyses were performed with the SPSS for Windows version 13 (SPSS Inc., Chicago, IL, USA). Distributions of continuous variables were assessed for normality using the Kolmogorov-Smirnov. Using univariate linear regression, path analysis and general liniar model were used to see the interaction of various variables on heart failure to determine the most influential variable on the heart failure. The results are presented in forms of narration and tables and graphs. For statistical tests, we set the level of significance at 5%.

Results

Table 1 describes the clinical and biochemical characteristics of the subjects. Table 2 and 3 showed correlation between variables and general linear model analysis of sTNFR I, MMP-9, PICP, and NT-proBNP. Figure 1 showed path analysis of sTNFR I, MMP-9, PICP, and NT-proBNP in heart failure.

The path analysis showed that there was a linear relationship between sTNFR I with MMP-9, R square was 25.8% (p = 0.00; r = 0.508), R square sTNFR I and MMP-9 with PICP was 14.4% (p = 0.038; r = 0.379) and R square MMP-9 and PICP with NT-proBNP was 39.6%(p = 0.00; r = 0.629). From all of the variable showed that the influence sTNFR I, MMP-9 and PICP to NT-proBNP of 61.7% and the influence of other variables that are not obtained from the model for 38.3%. Heart failure (based on NT-proBNP) more strongly influenced by inflammatory factors (sTNFR I) through a path of factor remodeling (MMP-9) and the fibrosis (PICP).

Figure 1 showed path analysis of sTNFR I, MMP-9, PICP, and NT-proBNP in heart failure.

Table 1. Clinical and Biochemical Characteristics of Heart Failure

Variables	Mean ± SD
Age (years)	61.4 ± 9.2
WC (cm	89.1 ± 9.0
SBP (mmHg)	120.9 ± 17.4
DBP (mmHg)	77.6 ± 10.7
EF (%)	61.3 ± 18.7
SGOT (U/L)	26.5 ± 14.7
SGPT (U/L)	26.0 ± 20.3
FPG (mg/dL)	109.5 ± 38.6
Total Cholesterol (mg/dL)	191.0 ± 36.5
Triglyceride (mg/dL)	120.3 ± 48.8
HDL Cholesterol (mg/dL)	46.1 ± 9.2
LDL Cholesterol (mg/dL)	125.8 ± 31.9
CCT (mL/min/1.73m^2)	48.8 ± 19.1
hsCRP (mg/dL)	2.2 ± 2.8
sTNFR I (pg/mL)	2.2 ± 1.1
NT-proBNP (pg/mL)	18.8 ± 1.4
MMP-9 (ng/mL)	495.2 ± 243.4
PICP (ng/mL)	73.5 ± 24.3

Description: WC = Waist Circumference; SBP = Systolic Blood Pressure; DBP= Diastolic Blood Pressure; EF = Ejection Fraction; SGOT = Serum Glutamic Oxalacetatic Transaminase; SGPT = Serum Glutamic Piruvate Transaminase; FPG = Fasting Plasma Glucose; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; CCT = Creatinine Clearance Test; hs-CRP = high sensitivity C-Reactive Protein; sTNFR I = soluble Tumor Necrosis Factor Receptor I; NT-proBNP = N-Terminal-pro Brain Natriuretic Peptide; MMP-9 = Matrix Metalloproteinase-9; PICP = Procollagen C-terminal Propeptide type I; SD= Standard Deviation.

Table 2. Correlation between variables

Variables	r	P
sTNFR I	0.474**	0.001
MMP-9	0.537**	0.000
PICP	0.488**	0.001

Description : sTNFR I = soluble Tumor Necrosis Factor Receptor I; MMP-9 = Matrix Metalloproteinase-9; PICP = Procollagen C-terminal Propeptide type I; NT-proBNP = N-Terminal-pro Brain Natriuretic Peptide.

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Table 3. Univariate Linear Regression and GLM Univariate Analysis

Variables	B	Coefficient B	SE	T	p	95% C.I	
						Lower	Upper
sTNFR I	1.098	0.206	0.747	1.471	0.149	-0.41	2.606
MMP-9	0.001	0.329	0.001	2.342	0.024	0.00	0.002
PICP	0.011	0.312	0.005	2.44	0.019	0.002	0.021

Description : sTNFR I = soluble Tumor Necrosis Factor Receptor I; MMP-9 = Matrix Metalloproteinase-9; PICP = Procollagen C-terminal Propeptide type I; NT-proBNP = N-Terminal-pro Brain Natriuretic Peptide.
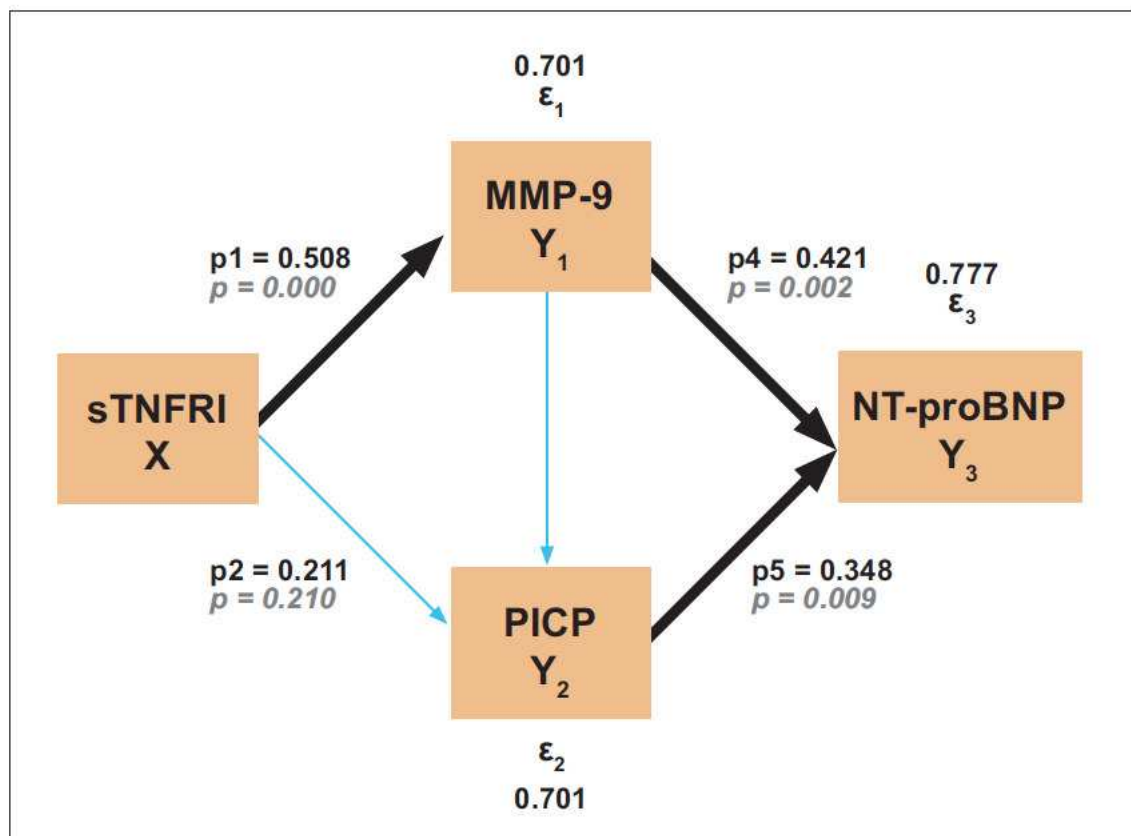

Figure 1. Path analysis Diagram. It appears from the drawing thick lines indicate significant pathways that can worsen the condition of heart failure.

Discussion

Analysis of Correlation between sTNFR I, MMP-9, PICP, and NT-proBNP in heart failure

This study showed that there was a significant positive correlation between sTNFR I with MMP-9 (r = 0.508 **, p = 0.000), and PICP (r = 0.326 * p = 0.029). It was clear that sTNFR I induced the secretion PICP and MMP-9, which associated with the development of heart failure.

Inflammation is important in the pathogenesis and progression of many forms of heart failure, and biomarkers of inflammation have become the subject of intense inquiry. According to the cytokine hypothesis of heart failure, proinflammatory cytokines (TNF-α, IL-1, IL-6, dan IL-18) are produced by the damaged myocardium; this production is enhaced by stimulation of the sympathetic nervous system. Injured myocardium, as well as skeletal muscle that is hypoperfused because of reduced cardiac output activates monocytes to produce the same cytokines, which act on and further impair myocardial function. The stressed myocardium releases natriuretic peptides to improve heart function. Expression of pro-inflammatory cytokines which exacerbate the effects associated with left ventricular function and accelerate the progression of heart failure (6).

Left ventricular (LV) remodeling is an important contribution event in the progression to end-stage congestive heart failure (CHF). MMPs and TIMPs are regulated at the transcriptional level by binding of transcription factors, enhancers and repressors to the gene's promoter region and their expression is modulated by external stimuli that are operative in cardiac disease states. MMP-9 has a nuclear factor-$\varkappa$B (NF-$\varkappa$B) binding site (7,8).

Binding of TNF induces the trimerization of the TNFR and the recruitment of a number of adaptor proteins. Kawamura *et al.* examined the effects of a double transgenic mouse model in which cardiomyopathy was induced by cardiac restricted overexpression of TNF and transcription was altered due to a disrupted p50 NF-$\varkappa$B subunit. With TNF overexpression only, MMP-9 was increased. However, with the TNF overexpression and disrupted NF-$\varkappa$B, the levels of MMP-9 were significantly attenuated (7).

It is accepted that myocardial fibrosis is the result of both increased collagen synthesis by fibroblasts and myofibroblasts and unchanged or decreased extracellular collagen degradation (9).

It is accepted that myocardial fibrosis is the result of both increased collagen synthesis by fibroblasts and myofibroblasts and unchanged or decreased extracellular collagen degradation (9).

Changes in the quantity and quality of the collagen network leads to changes in the structure and function of the left ventricle. Increased collagen, as shown in hypertrophy due to excessive pressure eventually increases stiffness of the wall of the myocardium and causes left ventricular hypertrophy. While degradation of the existing collagen as in myocardial infarction, reduces stiffness in the wall of the heart muscle and causes dilatation of the left ventricle. So both will lead to the abnormalities in left ventricular function (10,11).

Fibrotic tissue accumulation is an integral part of structural changes in heart tissue. PICP is Carboxy-terminal propeptide of type I and associated with collagen deposition in the myocardium in heart failure patients (9,12).

Univariate linear regression, path analysis, and general linear model of sTNFR I, MMP-9, and PICP in heart failure

From univariate linear regression and general linear model, we found that the greatest role of factors in heart failure was that of PICP and MMP-9, and based on path analysis the overall influence level of sTNFR I, MMP-9, PICP in the state of heart failure was 61.7% and that of the other factors outside the model was 38.3%.

Remodeling of extracellular matrix in the early stages of the process leading to heart failure. Collagen accumulation increased with the progression of hypertrophic changes in spite of enhanced MMP levels. It is not clear which event was initial, reactive fibrosis or MMP activation, as both were already present in patients with compensated hypertrophy and could be provoked by the same factors (13,14).

According to the study results based on this model, TNFR I induces MMP-9 as a factor in the remodeling and then the process of fibrosis supervened characterized by an increase in PICP. MMP may participate in fibrosis and remodeling process through digestion of matrix component and regulation of the formation matrikines such as glycil-histidyl-lysine and release of biologically active factors from the ECM (including TGFβ1, insulin-like growth factor, and fibroblast growth factor). Increase of sTNFRI, MMP-9 and PICP concentrations showed a poor prognosis in heart failure (15).

We realize that this study, however, does have limitations. In example, lack of consideration to subjects smoking, exercise and drug habits. Thus, a more advanced studies are needed, with a larger number of subjects; compared with a control groups; and also with attention to subjects smoking, exercise and drug habits.

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Discussion

From our study there were some important points. The prognosis of heart failure can be seen through examination of sTNFR I which is a receptor of TNF-α as a proinflamatory cytokines that cause remodeling factors (MMP-9) and fibrosis (PICP). Higher the concentration of each, referred to worse prognosis. From univariate linear regression and general linear model, MMP-9 and PICP may play roles in the association between sTNFR I and the increased risk of HF.

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