Correlation between Low Grade Inflammation with Endothelial Progenitor Cells Surface (EPCs) Marker in Hypertension

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Abstract
Patients with hypertension have been shown to express reduced number and functional capacity of endothelial progenitor cells (EPCs). Recent study reveals that EPCs contain an important capability to maintain endothelial integrity and vascular homeostasis. Therefore, enhancement of EPCs could be benefit for individuals with cardiovascular diseases. We investigated the correlation between low grade inflammation marker (hsCRP) with EPC surface marker (CD34 total) in hypertension. This was an observational study with cross sectional design conducted in 51 nonhypertenive and 60 hypertensive subjects. hsCRP (as marker of inflammation) was measured by immunochemiluminometric method. CD34 total (as marker of EPC) was measured by flow cytometry method. hsCRP concentration was higher in hypertensive subjects compare to nonhypertensive subjects (mean±SD, 2.709±2.50 vs 2.476±2.438 mg/L; P>0.05). CD34 total level was lower in hypertensive subjects compare to nonhypertensive subjects (mean±SD, 1.957±0.858 vs 3.244±3.463/μL; P<0.05). hsCRP had positive correlation with CD34 total in hypertensive subjects (P<0.05). The increasing concentration of hsCRP will stimulate the CD34 total level in hypertensive subjects as demonstrated by the positive correlation between hsCRP with CD34 total.

Key words: hsCRP, CD34 total, endothelial progenitor cell, hypertension

Korelasi antara Inflamasi Tingkat Rendah dengan *Endothelial Progenitor Cells Surface* (EPCs) *Marker* pada Hipertensi

Abstrak
Pasien dengan hipertensi menunjukkan penurunan jumlah dan fungsi kapasitas *Endothelial Progenitor Cells (EPCs)*. Penelitian terkini mengungkapkan bahwa EPCs memiliki kemampuan penting untuk memelihara integritas endotelial dan homeostasis pembuluh darah. Oleh karena itu, peningkatan kapasitas EPCs bermanfaat bagi individu dengan penyakit kardiovaskular. Penelitian ini menginvestigasi korelasi antara *marker* inflamasi tingkat rendah (hsCRP) dengan *marker* EPC *surface* (total CD34) pada hipertensi. Penelitian ini merupakan studi observasional dengan desain *cross sectional* pada 51 orang nonhipertensi dan 60 orang pasien hipertensi. hsCRP (*marker* inflamasi) diukur menggunakan metode *immunochemiluminometric*. Nilai total CD34 (*marker* EPC) diukur dengan menggunakan metode *flow cytometry*. Konsentrasi hsCRP lebih tinggi pada pasien hipertensi dibandingkan pada pasien nonhipertensi (*mean*±SD, 2,709±2,0 vs 2,476±2,438 mg/L; P>0,05). Nilai total CD34 lebih rendah pada pasien hipertensi dibandingkan dengan pasien nonhipertensi (*mean*±SD, 1,957±0,858 vs 3,244±3,463/μL; P<0,05). hsCRP memiliki korelasi positif dengan total CD34 pada pasien hipertensi (P<0,05). Peningkatan konsentrasi hsCRP akan menstimulasi level total CD34 pada pasien hipertensi seperti yang ditunjukkan dengan korelasi positif antara hsCRP dengan total CD34.

Kata kunci: hsCRP, total CD34, *endothelial progenitor cell*, hipertensi

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Introduction

Recent study indicates that endothelial injury in vascular wall is recovered by the colonization of the injured site of the vessel by endothelial progenitor cells (EPCs). EPCs have been de-monstrated in both animal and humans model to contribute to neovascularization and reendothelialization. EPCs are characterized by the expression of cell markers CD34, CD133 and Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) and by their ability to form endothelial integrity and vascular homeostasis.[1-3]

C-Reactive Protein (CRP) is considered as a biomarker for inflammation and as well a prominent partaker in endothelial dysfunction and atherosclerosis. CRP was found to be the stronger predictor of incident cardiovascular events. CRP is an appropriate marker because it has a long half-life and remains stable over time without exhibiting circadian variability.[4] High sensitivity CRP has emerged as an independent predictor of cardiovascular disease risk.[5]

On the other hand, inflammation is actively implicated in the pathophysiology of cardiovascular disease. Proinflammatory cytokines trigger the synthesis of acute phase proteins in the liver while they also stimulate the expression of adhesion molecules on endothelial surface, promoting in atherogenesis The inflammatory molecules are elaborated soon after ischemic injury and stimulate production of growth factors and mediate tissue repair and adaptation. EPCs are released in peripheral circulation after cytokine stimulation and take part in this process.[1] In this present study, the study isinvestigate the correlation between low grade inflammation, which is represented by hsCRP and EPCs which is represented by CD34 total in non hypertension patients.

Methods

This was an observational study with cross sectional design conducted in 60 subjects with hypertension, age between 35–60 years old. Patients that fulfill inclusion and exclusion criteria were given informed consent to participate in this study. Hypertension was diagnosed according to Joint National Committee (JNC) VII, if systolic and diastolic blood pressure was equal to or higher than 140 and 90 mmHg, respectively, on two or more visit at 1 week interval. Patients with blood pressure below criteria were classified as nonhypertensive patients. Excluded from the study was the participant having overt diabetic disease at the moment of blood collection. Participants receiving antihypertensive drug treatment were also excluded. The study protocol was approved under Hasanuddin University Ethics Committee (*Unhas Nomor*: UH09010006) and all participants agreed to give their consent to participate in this study.

Assay of Biochemical Profile

Venous blood was collected from all subjects following 10–12 hours fasting and serum was separated from whole blood after centrifugation and immediately kept at -20 ºC until measurement. Tryglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and glucose were determined by an enzymatic-colorimetric method (Advia 1800; Siemens Healthcare Diagnostics Inc). hsCRP was determined by immunochemiluminometric method (Immulite 2000; Siemens Healthcare Diagnostics Inc). CD34 total was determined by flow cytometry method (FACSCalibur, BD Biosciences).

Statistical Analysis

Statistical analysis was performed using SPSS version 13.00. Values were expressed as the mean±SD. Mann Whitney was used to compare variables between nonhypertensive subjects and hypertensive subjects. Spearman's coefficient of correlation was used to determine correlation between study variables.

Results

The characteristics of 51 nonhypertensive subjects and 60 hypertensive subjects were summarized in Table 1.

Table 1 Subject characteristics

	Nonhypertensive (N=51)	Hypertensive (N=60)	P
Age (Years)	47 ± 9	51 ± 8	< 0.01
Waist circumference (cm)	87 ± 10	91 ± 9	< 0.05
Sistolic BP (mmHg)	127 ± 5	142± 11	< 0.01
Diastolic BP (mmHg)	81 ± 4	92 ± 7	< 0.01
LDL Colesterol (mml/L)	138.4 ± 30	135.6 ± 33	< 0.05
HDL Colesterol (mml/L)	47 ± 9	50 ± 10	< 0.05
Triglycerides (mml/L)	132 ± 60	139 ± 65	< 0.05
Fasting Glucose (mml/L)	95 ± 7	96 ± 12	< 0.05

BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein. Values were displayed in mean ± SD.

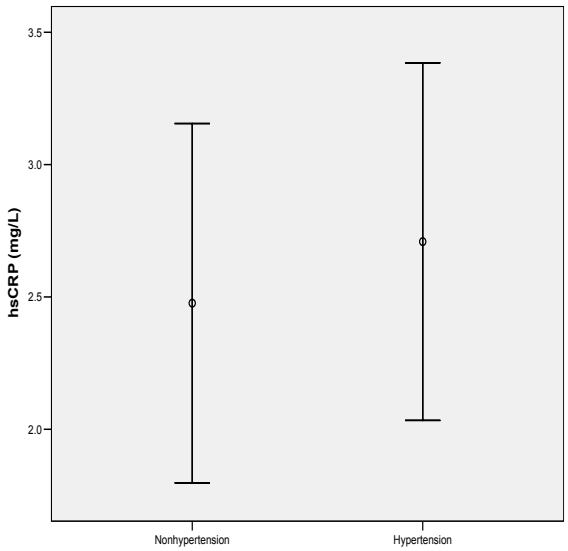

Figure 1 Difference concentration of hsCRP in nonhypertensive and hypertensive subject

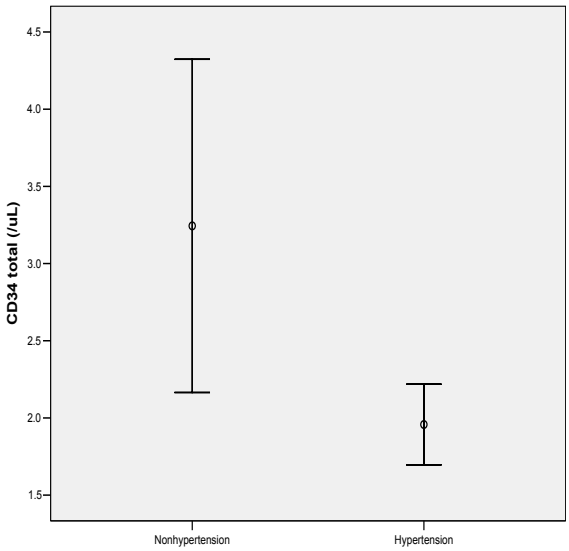

Figure2 Difference concentration of CD34 total in nonhypertensive and hypertensive subject

hsCRP was slightly higher in hypertensive subjects compared to nonhypertensive subjects (mean±SD, 2.709±2.50 vs 2.476±2.438 mg/L; p>0.05). CD34 total was lower significantly in hypertensive patients compare to nonhypertensive patients (mean±SD, 1.957±0.858 vs 3.244±3.463/µL; p<0.05). hsCRP had positive correlation with CD34 total (p<0.05).

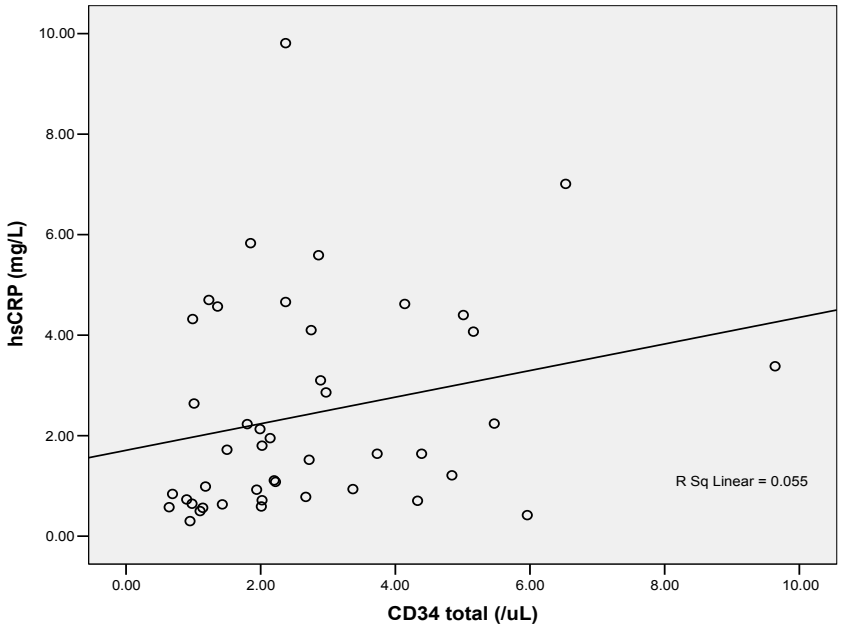

Figure 3 Correlation between hsCRP concentration and CD34 total level

Discussion

The main finding of our study was that increased low grade inflammation would lead to the increase of EPCs number in hypertensive subjects. This hypothesis was supported by our observation of a positive correlation between hsCRP concentration and CD34 total level.

EPCs are rapidly mobilized after vascular trauma, in response to a rise in circulating VEGF levels that contribute to the revascularization of the injured tissues. Inflammatory molecules have been demonstrated to induce EPC mobilization from the bone marrow. Recently, both in vitro and in vivo studies, microvascular endothelial cells challenged with inflammatory stimuli expressed the membrane-bound of KitL and recruited EPCs via c-Kit mediated activation of the Akt signaling pathway.[6-8] This evidence showed that restricted inflammatory response may constitue a stimulus for EPC mobilization.

In our cross-sectional study, we found that, low grade inflammation in hypertension will induce EPCs mobilization. This study in line with other studies showed by Guven et al, that in patients with Coronary Artery Disease (CAD), the number of EPCs seems to be increased in association with angiographically significant CAD.[9] George et al, showed a positive correlation between CRP levels and circulating EPCs has been documented in patients with stable coronary artery disease, suggesting that a systemic infrlammatory state could potentially stimulate EPC mobilization in these individuals.[10]

Conclusions

The increasing concentration of low grade inflammation will stimulate the EPCs level in hypertensive patients as demonstrated by the positive correlation between hsCRP and CD34 total in hypertensive patients. We hypothesized that hypertension-induced low grade inflammation will contribute to the increase of EPC levels.

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