

RESEARCH ARTICLE

The Number and Potency of Endothelial Progenitor Cells in Type 2 Diabetes Mellitus Patients

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Abstract

BACKGROUND: Type 2 Diabetes Mellitus (T2DM) is a metabolic disease, due to the disorder of insulin function, insulin secretion, or both. Long-term hyperglycemia conditions promote endothelial dysfunction precedes to the development of multiple organ dysfunctions. Endothelial progenitor cells (EPCs) and hematopoietic stem cell (HSCs) are the key cellular effectors of postnatal neovascularization and play central role in endothelial dysfunction. However, in T2DM condition, the number of apoptotic HSCs increase, it may cause the reduction in potency and number of EPCs. In diabetes, the circulating EPCs number decrease and their functionality is impaired, but mechanism underlie of this impairment is unknown. The purpose of this study was to examine the relationship duration diabetes with the number and potency of EPC cells in T2DM patients controlled and poorly controlled.

METHODS: Thirty-eight T2DM male patients were classified into two group based on Indonesian Society of Endocrinology/Perkumpulan Endokrinologi Indonesia (PERKENI) criteria on T2DM. The first group was

a controlled glycemic condition group (hemoglobin A1c (HbA1C) <7.0%) and the second group was a poorly controlled glycemic condition group (HbA1C >7.0%). Cluster of differentiation (CD)34⁺ and CD133⁺ expressions were used as specific marker for EPC, while quantified bright aldehyde dehydrogenase (ALDH^{br}) assay was used to represented the potency of EPCs.

RESULTS: This study showed that in poorly controlled T2DM group the number of EPCs was lower by 24.80% ($p<0.05$) compared to the T2DM controlled group. Similarly, the expression of ALDH^{br} was lower by 43.07% ($p<0.05$) in poorly controlled group.

CONCLUSION: There was a decrease in the number and potency of EPCs in poorly controlled T2DM patients compared to the controlled T2DM patients. There was also a strong negative correlation between the duration of diabetes and number of EPCs.

KEYWORDS: ALDH^{br}, endothelial progenitor cells, type 2 diabetes mellitus

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a metabolic disease, characterized by elevated levels of blood glucose, due to the impair insulin function, insulin secretion, or both. Usually in adults, it occurs when the body becomes resistant to insulin, or also known as insulin resistance. The prevalence of this

disease has risen every year.(1) Long-term hyperglycemia conditions results in glucotoxicity by forming advanced glycosylation end products (AGEs), which contribute to the increased oxidative stress.(2) Long-term hyperglycemia also promotes endothelial dysfunction which precedes to the development of multiple organ dysfunctions.

Oxidative stress overwhelms the defense mechanisms of the vascular endothelium, which followed by an increase

of endothelial vasoconstriction, adhesion, inflammatory and pro-thrombotic molecules which results in endothelial damage. These conditions trigger body compensation to make improvements in maintaining vascular homeostatic and angiogenesis, by involving the proliferation and mobilization of immature cells for angiogenesis and covering the endothelial damage.(3)

Circulating endothelial progenitor cells (EPCs) may play a major role in the capacity of vascular repair. Recent evidence, EPCs can proliferate, migrate and differentiate into endothelial cells to maintain the endothelial integrity and hemostasis.(4) In diabetes patients, hyperglycemia increases reactive oxygen species (ROS) and AGEs production which may impair the EPC function. EPC number and function have been shown to be significantly diminished in diabetic patients compared to healthy controls. The mechanism underlying this decrease in number and functionality impairment are still poorly understood.(5)

Experimental studies have shown that high glucose concentrations exposure EPC to increase superoxide anions and reduce bioavailability of nitric oxide (NO). EPC dysfunction, which may be involved in the pathogenesis of vascular complications due to endothelial insufficiency in the process of vascular generation.(6,7) Physiologically, EPC has a role in revascularization through the enzyme aldehyde dehydrogenase (ALDH), an enzyme that catalyzes the pyridine nucleotide associated with aldehyde oxidation, into carboxylic acid, which converts acetaldehyde to acetyl acid, as energy so that cells can carry out cell functions in development, increase and revascularization. ALDH beam measurements are the measurements of light. For example is bright ALDH (ALDH^{br}) expresseion in cells describes the cell's potential and representatively predict the cell's capacity to proliferate and engraftment.(8)

The aim of this study was to determine the number of circulating EPC and potency of EPCs in controlled and poorly controlled T2DM patients, thereby providing an additional information regarding the relationship of EPC with vascular complications in T2DM.

Methods

Subjects

This was an observational cross-sectional study held in Jakarta during December 2017. The ethical approval was obtained from The Ethics Commission of Faculty of Medicine, Universitas Padjadjaran (No. 1027/UN6.C.10/

PN/2017). Thirty-eight male T2DM male subjects with T2DM were enrolled in this study. All subjects had given written informed consent before participating.

Subjects' clinical history, medications, and their glycosylated hemoglobin (HbA1C) results were reviewed. Controlled and poorly controlled patients were grouped based on the T2DM criteria determined by Indonesian Society of Endocrinology/Perkumpulan Endokrinologi Indonesia (PERKENI) in 2015. Subjects with HbA1C <7.0% were grouped into controlled glycemic condition group and subjects with HbA1C >7.0% were group into poorly controlled glycemic condition group. There was no subjects with coronary artery disease and hypertension.

HbA1c Test

The venous blood samples were collected and the examined to find the concentration of HbA1c by using high performance liquid chromatograph (HPL-C)-ion exchange method (Variant, Biorad, California, USA). The concentration was expressed in units of percent (%).

Quantification of EPC Numbers and Cell Potential

To analyze the number and potency of EPCs, the cell sorting process was carried out using the flow cytometry method (BD FACSCANTO II, Bioscience, California, USA). The number of EPCs was identified with surfaces markers with staining, by counting the expression of cluster of differentiation (CD)34⁺ (CD34 Antibody, Miltenyi Biotec, Bologna, Italy) and CD133⁺ (CD133 Antibody, Miltenyi Biotec) antigen surfaces.

The evaluation of potency EPCs was done using the ALDEFLOUR kit (Catalogue #01700, Stemcell Technology, Vancouver, Canada). One mL ALDEFLOUR assay buffer, 5 µL ALDEFLOUR DEAB, and 5 µL ALDEFLOUR was added to the sample (cell suspension). Sample was incubated for 45 minutes and centrifuged for 5 minutes. Then the supernatant was discarded and the pellet was added with 0.1 mL ALDEFLOUR buffer assay and transferred to the trucount tube. The cocktail mixed antibodies were added to the sample, followed by 20 minutes incubation at 4°C.

Statistical Analysis

The data were analyzed using SPSS 24 software (SPSS Inc., Chicago, USA) for Windows operating systems. The standard error used was 5%. The data obtained were tested with the Shapiro-Wilk method to determine the distribution pattern. It was further tested with Spearman correlation test and Mann-Whitney U-test.

Table 1. Study subjects characteristics.

	Controlled (n=8)			Poorly Controlled (n=30)			<i>p</i> value
	Median	Percentile 5	Percentile 95	Median	Percentile 5	Percentile 95	
Age (years)	52	32	63	55	34	65	0.52
Duration of diabetes (years)	3	1	23	4	1	16	0.58
HbA1c (%)	6.7	5.8	6.9	9.6	7	14.7	0.00*
EPC (cell/mL)	60.75	6.88	798.4	13.43	0	156.83	0.04*
EPC + ALDH ^{br}	60.75	6.88	249.5	13.43	0	153.26	0.03*

Results

From the results of this study, we obtained an abnormal distribution pattern. The age range of subjects who participated in this study was 31-68 years old, meanwhile the duration since they were diagnosed with T2DM was varied from 1-27 years. There were 8 subjects classified as the controlled T2DM patients and 30 subjects classified as the poorly controlled. The subjects' characteristic comparison between the controlled and poorly controlled T2DM group were presented in Table 1.

From the characteristics of the subjects, there was no significant difference in age and duration of diabetes between the two groups of T2DM patients. It illustrated that the age and duration of diabetes do not distinguish the T2DM group from being controlled or poorly controlled. In the poorly controlled T2DM group, the number of circulating EPC was lower compared to the controlled

group (Figure 1). The median number of circulating EPC in controlled group was significantly higher than the poorly controlled group (60.75 vs. 13.43, $p=0.04$).

The EPCs number carried out in the study showed a difference between poorly controlled and controlled groups, where the number of circulating EPC in the significantly poorly controlled group decreased compared to the controlled group, as also presented in Figure 2. The numbers of circulating EPCs, was determined by the co-expression of CD34⁺ and CD133⁺, with the area of getting low side scatter vs. forward scatter (SSC/FSC), which was quantified by using flow cytometry.

Furthermore, ALDH^{br} expression in the cells were also shown in getting low scatter slides (Figure 3). In T2DM patients, the number of EPC that given ALDH^{br} was significantly lowered in poorly controlled group compared to the controlled group ($p<0.05$). Based on the Spearman's correlation analysis, the number of circulating EPC were positively correlated with the number of

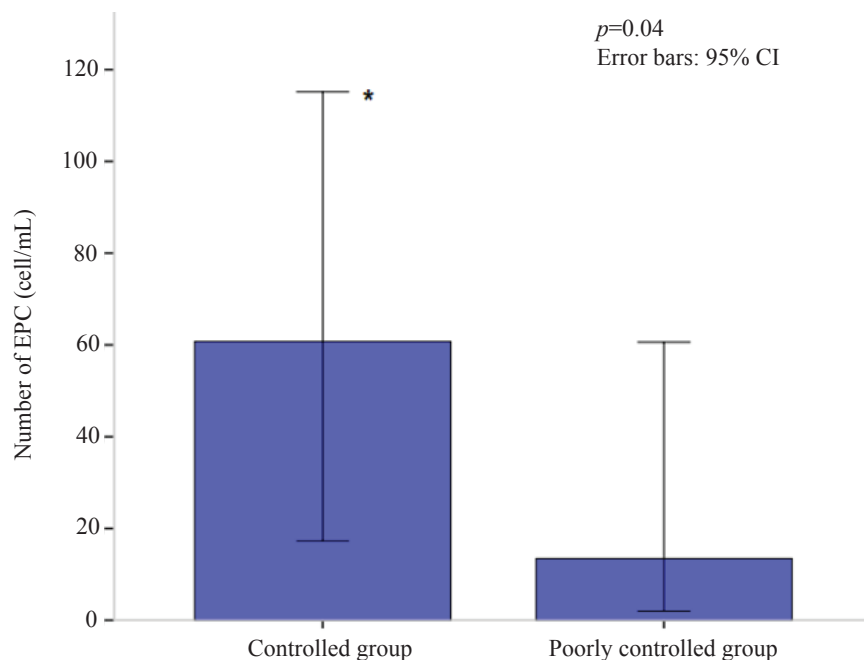


Figure 1. Number of circulating EPC in the controlled group and poorly controlled group.

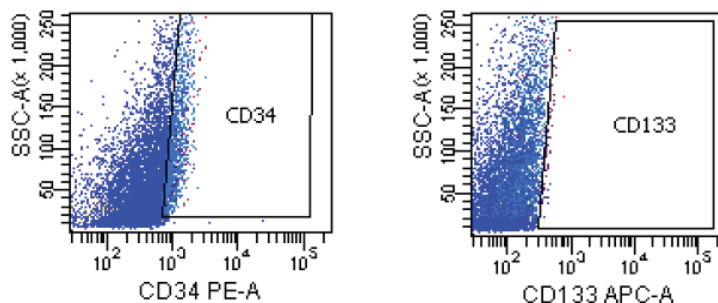


Figure 2. EPC numbers were determined by flow cytometry for the co-expression of CD34⁺ and CD133⁺.

EPC that given ALDH^{br} ($r=0.99, p<0.05$), as well inversely associated with the duration of diabetes ($r=-0.37, p<0.05$). The activity of ALDH^{br} in EPCs was around 55.5% and 96% for controlled and poorly controlled group, respectively.

There was no correlation between HbA1c and the number of EPC, but there was a strong negative correlation ($r=-0,347, p=0.03$) between the duration of diabetes and the number of EPC, as well as the duration of diabetes and number of EPC + ALDH^{br} ($r=-0.367^*, p=002$).

Discussion

It was found that EPC number was higher in the controlled group compared to the poorly controlled group. The results of this study are in line with a study conducted in 2010, which showed a significantly lower number of EPC (CD34⁺ EPC) between poorly controlled T2DM compared to healthy group, which was 0.767 ± 0.18 and 1.033 ± 0.27 , with $p=0.203$.(7) Uncontrolled long-term hyperglycemia conditions contribute to an increased oxidative stress and AGE which results in a decrease in the number of circulating EPC.(2) Furthermore, uncontrolled ROS conditions are a significant trigger in the stem/progenitor cell-related aging, which can lead to an inactivation of telomerase.(9-11)

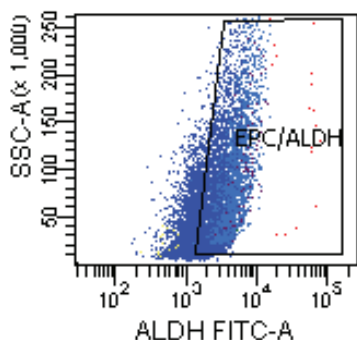


Figure 3. Getting area of EPC that express ALDH^{br}.

The positive correlation between EPC and EPC + ALDH^{br} in this study illustrates that high ALDH activity elevated with increasing number of EPC. Significant differences of EPC added by ALDH between controlled and poorly controlled groups is that the expression of ALDH is higher in low ROS conditions.(12,13) ALDH is a family of 19 cytosolic enzymes that can catalyze pyridine which is associated with the oxidation of aldehydes to carboxylic acids.(9) ALDH plays a role in the oxidation process of intracellular aldehydes and oxidation of retinol into retinoic acid during the early stages of stem cell development which has pro/anti-angiogenic effects for endothelial cell cytokine modulation, migration and vasculogenesis effects. (14) ALDH shows the potency of progenitor cells, which are related to its function and colonization. A previous study suggested that ALDH^{br} cells are predictive of engraftment, which would be expected because this enzyme is characteristically highly expressed in the hematopoietic stem and progenitor cells from human umbilical cord blood, bone marrow, and mobilized peripheral blood.(8)

Interestingly, in this study, there was no correlation between HbA1c and the number of EPC, but there was a strong negative correlation between the duration of diabetes and a decrease in the number of EPC. This result is consistent with the another study conducted in 2015 which reported an insignificant association between circulating CD34⁺ cell counts and HbA1c levels in T2DM patients.(15) Likewise, it was stated that in patients with duration of diabetes >10 years, CD34⁺ cell counts were lower than patients with duration of diabetes <10 years.(3)

Results of this study shows that the exposure to hyperglycemia contributes to reducing the number and potential of EPC cells. The lower number of EPC in the peripheral blood circulation in the poorly controlled group is related to impaired mobilization from hematopoietic stem cell (HSC) and EPC, reflecting a disturbed niche function due to diabetes conditions.(16) Mobilization disorders begin with increased oxidative stress condition, pro-inflammatory

factors and apoptosis which cause vascular complications. (3) The high ROS accumulation can cause stem cell-related aging which lead to HSC and EPC mobilization. (10,17,18). A decrease in the number of progenitor cells from the spinal cord is characterized by a lower number of cells in circulation. This is caused by disruption of progenitor cell mobilization and increased apoptosis associated with long-term diabetes exposure. (3)

Conclusion

There was a decrease in the number and potency of EPC in poorly controlled T2DM patients compared to the controlled T2DM patients and also a strong negative correlation between the duration of diabetes and number of EPC. Further studies of EPC and EPC with ALDH^{br} as a marker to detect cardiovascular disease risk in diabetics is needed.

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