中文題目：在酒精性脂肪肝之病人, 血管內皮前驅細胞數量及功能減少的研究

英文題目：Decreased Circulating Endothelial Progenitor Cell Levels and Function in Patients with Nonalcoholic Fatty Liver Disease

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Background:

Nonalcoholic fatty liver disease (NAFLD) is associated with advanced atherosclerosis and a higher risk of cardiovascular disease. Increasing evidence suggests that injured endothelial monolayer is regenerated by circulating bone marrow derived-endothelial progenitor cells (EPCs), and levels of circulating EPCs reflect vascular repair capacity. However, the relation between NAFLD and EPC remains unclear. Here, we tested the hypothesis that patients with nonalcoholic fatty liver disease (NAFLD) might have decreased endothelial progenitor cell (EPC) levels and attenuated EPC function.

Materials and Methods:

34 patients with a diagnosis of NAFLD diagnosed by performing abdominal ultrasonography, as the study group, and 68 age- and gender-matched patients without ultrasonographic NAFLD, as controls, were enrolled in this study.

Assay of circulating EPCs

A volume of 1000-μL peripheral blood was incubated for 30 minutes in the dark with monoclonal antibodies against human KDR followed by APC-conjugated secondary antibody, with the FITC-labeled monoclonal antibodies against human CD45, with the PE-conjugated monoclonal antibody against human CD133, and with FITC-conjugated monoclonal antibodies against human CD34. After incubation, cells were lysed, washed with phosphate-buffered saline, and fixed in 2% paraformaldehyde before analysis. Each analysis included 100,000 events. As shown in Figure 1, the numbers of circulating EPCs were gated with monocytes and defined as $CD34^{+}CD45^{low}$, $CD34^{+}KDR^{+}CD45^{low}$, and $CD34^{+}KDR^{+}CD133^{+}CD45^{low}$, respectively.

EPC characterization

The early EPCs were characterized as adherent cells double positive for acetylated low-density lipoprotein uptake and lectin binding by direct fluorescent staining as previously described. Briefly, the adherent cells were first incubated with 2.4 μg/ml DiI-acLDL for 1 hour and then fixed in 2% paraformaldehyde and counterstained with 10 μg/ml FITC-labeled lectin from Ulex europaeus. The late EPC-derived

outgrowth endothelial cell population was also characterized by immunofluorescence staining for the expression of VE-cadherin, platelet/endothelial cell adhesion molecule-1, CD-31, and CD34. The fluorescent images were recorded under a laser scanning confocal microscope. Fibronectin adhesion test and migration test were also done.

Results

Clinical and laboratory data

The mean age of the 102 study patients (48 males, 47%) was 70 ± 14 years. The patients with NAFLD in the study group and those without NAFLD in the control group were matched for age and gender. The baseline characteristics of all study subjects are presented in Table 1. No significant differences were noted between the 2 groups except metabolic syndrome, hyperuricemia, and previous history of myocardial infarction.There were no significant differences between the 2 groups in terms of currently used medications. In addition, patients with NAFLD had significantly higher waist circumference and BMI values, as well as increased plasma uric acid and lower HDL-C levels than those without NAFLD (Table 2).

Circulating EPC levels

As shown in Table 3, NAFLD patients had significantly decreased levels of circulating EPCs. For further analysis, subjects with NAFLD were divided into 3 groups according to the severity of fatty liver in ultrasonographic analysis: group 1, mild fatty liver (n = 17); group 2, moderate fatty liver (n = 10), and group 3, severe fatty liver (n = 7). Circulating EPC numbers were negatively associated with the severity of fatty liver (Figure 2).

Characterization of human EPC and functions

The peripheral blood MNCs that initially seeded on fibronectin-coated wells were round in shape (Figure 3A). After the medium was changed on day 4, attached early EPCs appeared to be elongated with a spindle shape (Figure 3B). Late EPCs with a cobblestone-like morphology similar to mature endothelial cells were grown to confluence (Figure 3C). Late EPC characterization was performed by immunohistochemical staining, and most of the cells expressed mature endothelial markers, VE-cadherin, PECAM-1 (CD31), and CD34 (Figure 3D-E), which are considered critical markers of late EPCs. Patients with NAFLD showed attenuated EPC adhesive function in comparison to those without NAFLD (controls vs. NAFLD, 32.6 ± 6.3 vs. 15.4 ± 5.7 cells/HPF, $P < 0.001$; Figure 4A). Moreover, Patients with

NAFLD had impaired EPC migration in comparison to those without NAFLD (controls vs. NAFLD, 56.5 ± 6.9 vs. 27.4 ± 8.1 cells/HPF, $P < 0.001$; Figure 4B).

Patients with NAFLD had significantly higher plasma concentrations of hsCRP(control vs. NAFLD: 0.96 ± 0.96 vs. 1.93 ± 1.70 mg/L, $P = 0.013$).

Independent correlates of nonalcoholic fatty liver disease

As shown in Table 4, using univariate analysis, reduced circulating EPC levels ($CD34^+$, $CD34^+/KDR^+$, and $CD34^+/KDR^+/CD133^+$), metabolic syndrome, uric acid, and hsCRP were found to be significant predictors of NAFLD. After adjustment for metabolic syndrome and uric acid levels, circulating EPC level ($CD34^+/KDR^+$) was still an independent negative predictor of NAFLD.

Conclusions

This study demonstrated for the first time that NAFLD patients have decreased circulating EPC numbers and adhesive function than those without NAFLD. These findings suggest that attenuated endothelial repair capacity may contribute to atherosclerotic disease progression and enhanced cardiovascular risk in NAFLD patients. NAFLD should be carefully considered as an independent risk factor for cardiovascular diseases.

Table 1. Baseline characteristics of study subjects

	No fatty liver (n = 68)	Fatty liver (n = 34)	P value
Age (years)	70 ± 13	71 ± 15	0.191
Male, n (%)	32 (47)	16 (47)	1.000
Hypertension, n (%)	56 (82)	29 (85)	0.925
Type 2 diabetes mellitus, n (%)	26 (38)	16 (47)	0.522
Metabolic syndrome, n (%)	28 (41)	24 (71)	0.010
Coronary artery disease, n (%)	39 (57)	25 (74)	0.169
Peripheral artery disease, n (%)	15 (22)	12 (35)	0.234
Chronic kidney disease, n (%)	27 (40)	17 (50)	0.364
Hyperlipidemia, n (%)	37 (54)	21 (62)	0.621
Current smoker, n (%)	12 (18)	9 (27)	0.311
Previous myocardial infarction, n (%)	14 (21)	15 (44)	0.024
Previous cerebrovascular disease, n (%)	10 (15)	6 (18)	0.923
Atrial fibrillation, n (%)	11 (16)	7 (21)	0.783
Hyperuricemia, n (%)	18 (27)	26 (77)	<0.001

Values are mean ± standard deviation (SD) or number (%)

Table 2. Metabolic profiles and medications of study subjects

	No fatty liver (n = 68)	Fatty liver (n = 34)	P value
Waist circumference (cm)	84.4 ± 9.3	89.8 ± 7.8	0.005
BMI (kg/m^2)	24.6 ± 4.0	26.7 ± 5.3	0.029
Cholesterol (mg/dL)	168 ± 48	175 ± 38	0.406
LDL-C (mg/dL)	98 ± 45	106 ± 32	0.366
HDL-C (mg/dL)	47 ± 13	41 ± 12	0.048
Triglyceride (mg/dL)	119 ± 62	143 ± 90	0.115
Creatinine (mg/dL)	1.8 ± 2.3	1.9 ± 2.0	0.972
Total bilirubin (mg/dL)	0.5 ± 0.2	0.6 ± 0.4	0.291
ALT (U/L)	23 ± 23	35 ± 37	0.055
γGT (U/L)	39 ± 65	47 ± 60	0.723
Uric acid (mg/dL)	5.9 ± 1.8	7.9 ± 2.5	<0.001
Fasting glucose (mg/dL)	135 ± 62	136 ± 59	0.933
HbA1c (%)*	6.9 ± 0.6	7.1 ± 0.7	0.370
Medication			
Aspirin, n (%)	43 (63)	23 (68)	0.826
Clopidogrel, n (%)	33 (49)	18 (53)	0.834
ACEI, n (%)	9 (13)	3 (9)	0.744
ARB, n (%)	25 (37)	12 (35)	1.000
CCB, n (%)	31 (46)	15 (44)	1.000
Beta blockers, n (%)	27 (40)	15 (44)	0.831
Diuretics, n (%)	23 (34)	13 (38)	0.826
PPAR-γ agonists, n (%)	10 (15)	8 (24)	0.409
Statins, n (%)	34 (50)	16 (47)	0.944
Nitrates, n (%)	32 (47)	20 (59)	0.363
Metformin, n (%)	13 (19)	8 (24)	0.612
Insulin , n (%)	7 (10)	5 (15)	0.528

Values are mean ± standard deviation (SD) or number (%)

*HbA1c levels of type 2 diabetes patients

Table 3. Comparison of the levels of circulating endothelial progenitor cells (EPCs), inflammatory markers, and ADMA in fatty liver patients versus controls

	No fatty liver (n = 68)	Fatty liver (n = 34)	P value
EPC levels (%)			
$CD34^+$	0.093 ± 0.093	0.029 ± 0.029	0.003
$CD34^+KDR^+$	0.024 ± 0.016	0.005 ± 0.005	<0.001
$CD34^+KDR^+CD133^+$	0.021 ± 0.016	0.005 ± 0.004	<0.001
EPC levels (cells/μl)			
$CD34^+$	132.2 ± 121.6	39.7 ± 43.7	0.008
$CD34^+KDR^+$	32.4 ± 22.4	8.8 ± 8.3	<0.001
$CD34^+KDR^+CD133^+$	27.1 ± 20.7	7.4 ± 7.2	<0.001
hsCRP (mg/L)	0.96 ± 0.96	1.93 ± 1.70	0.013
ADMA (μmol/L)	0.66 ± 0.49	0.78 ± 0.42	0.269

Values are mean ± standard deviation (SD)

hsCRP: high sensitivity C-reactive protein; ADMA: asymmetric dimethylarginine

Table 4. Simple correlation and multivariate analysis of factors associated with nonalcoholic fatty liver disease

	OR (95% CI)	P value
Univariate analysis		
EPCs (cells/μl)		
$CD34^+$	0.97 (0.96–0.99)	<0.001
$CD34^+KDR^+$	0.83 (0.76–0.90)	<0.001
$CD34^+KDR^+CD133^+$	0.82 (0.74–0.90)	<0.001
Age	1.11 (0.81–2.57)	0.805
Male	1.13 (0.49–2.57)	0.778
Hypertension	1.23 (0.39–3.88)	0.728
Diabetes	1.44 (0.62–3.36)	0.391
Hyperlipidemia	1.29 (0.56–3.03)	0.555
Metabolic syndrome	3.90 (1.59–9.55)	0.003
Peripheral artery disease	1.91 (0.76–4.79)	0.169
Coronary artery disease	2.22 (0.90–5.52)	0.085
Chronic kidney disease	1.74 (0.75–4.05)	0.200
Current smoking	1.68 (0.63–4.50)	0.302
Uric acid (mg/dL)	1.61 (1.25–2.08)	<0.001
hsCRP (mg/L)	1.79 (1.07–2.97)	0.026
ADMA (μmol/L)	1.78 (0.61–5.18)	0.293
*Multivariate analysis		
EPCs (cells/μl) $CD34^+KDR^+$	0.85 (0.78–0.93)	<0.001

*Multivariate analysis: adjusted for metabolic syndrome and uric acid levels.

Figure legends

Figure 1. Representative flow cytometry analysis for quantifying the number of circulating endothelial progenitor cells (EPCs).

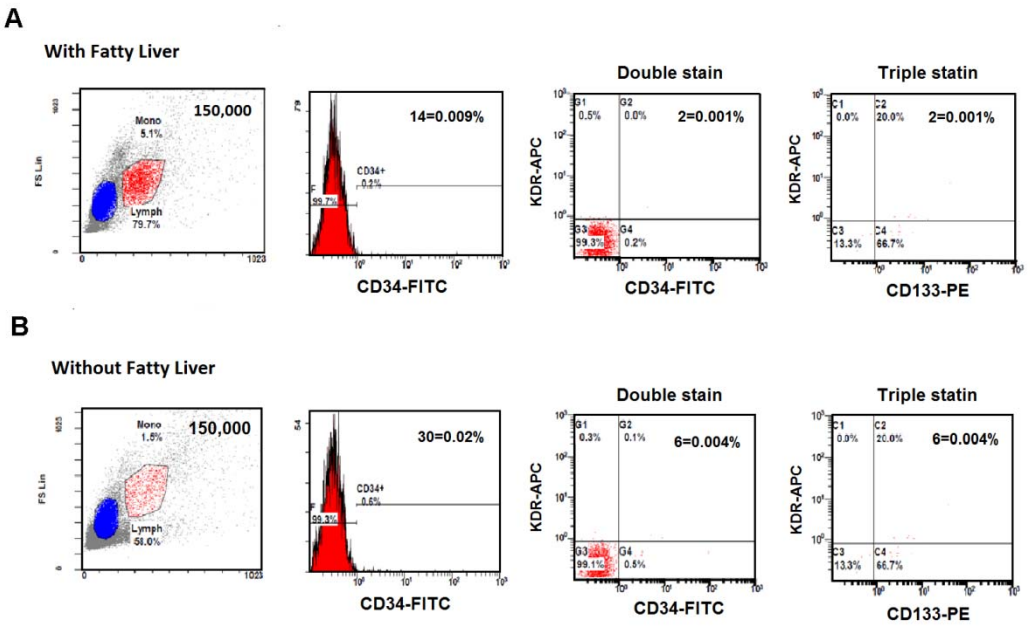

Figure 2. The association between EPC levels (% and cells/μl) and the severity of non-alcoholic fatty liver disease (values presented as means ± standard error; FL, fatty liver; Mild, mild fatty liver; Moderate, moderate fatty liver; Severe, severe fatty liver)

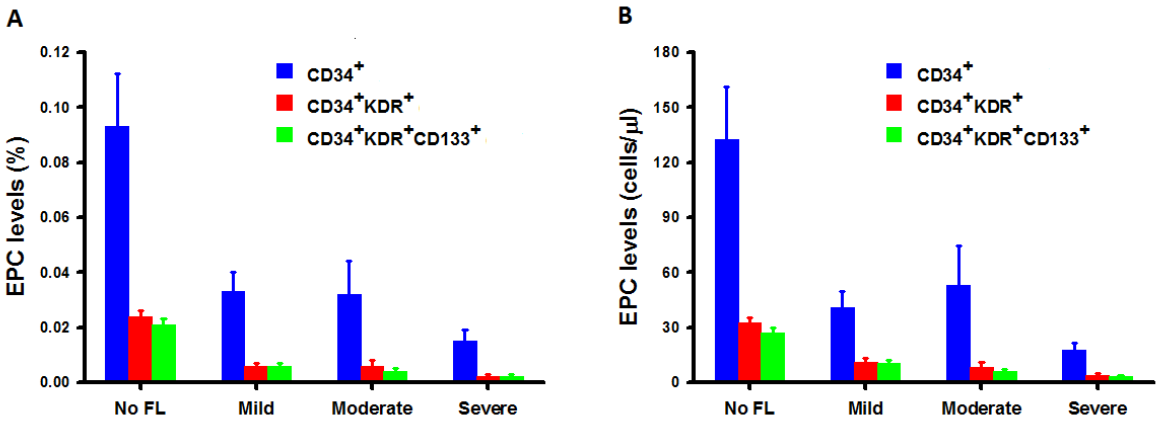

Figure 3. Morphology and characterization of human endothelial progenitor cells (EPCs) from peripheral blood.

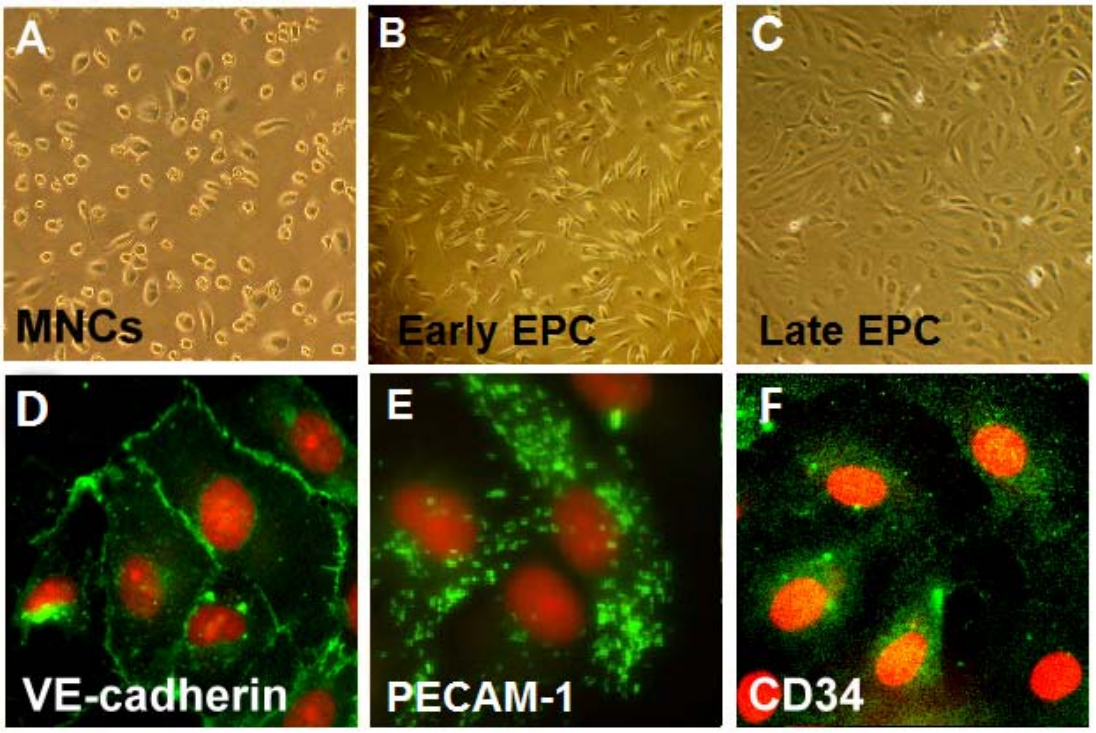

Figure 4. Comparison of the EPC adhesive function (A) and migration (B) in subjects with or without fatty liver (values presented as means ± SD; HPF: high-power field; *P < 0.05)