Analysis of peripheral blood biochemical parameters in diabetic retinopathy

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Analysis of peripheral blood biochemical parameters in diabetic retinopathy

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The Master's Thesis
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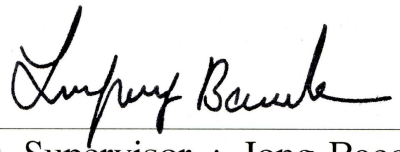
This certifies that the Master's Thesis
of Jonghan Lee is approved.



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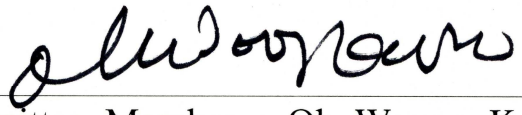

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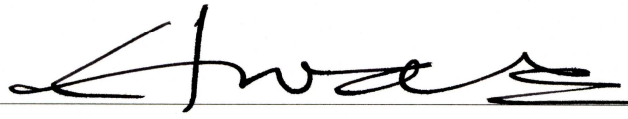

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I think climbing a mountain is hard enough but it gives refreshment, a sense of achievement and happiness of life.

This Master thesis gave me such a valuable learning experience and reminded me of some precious life priniciples.

I started to study laboratory medicine three years ago. I think progress of laboratory medicine is so fast and there are many interesting research fields. I want to study more for learning research experiences with a broader perspective.

Most of all, I praise God for his everlasting goodness and faithfulness.

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I give my best regard to my honorable parents who raised me with endless love. I also deeply appreciate my beloved wife.

I hope I keep on dreaming and any dream will do.

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ABSTRACT

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Introduction: To evaluate the clinical usefulness of peripheral blood biochemical parameters for the prediction of diabetic retinopathy (DR), peripheral blood levels of various biochemical parameters were measured in diabetes mellitus (DM) patients with or without diabetic retinopathy (DR).

Materials and Methods: The peripheral blood samples were obtained from 98 DM patients and 41 healthy controls. There were 38 DM patients without DR and 60 with DR, and the DR cases were categorized as non-proliferative DR (NPDR) in 51 patients and proliferative DR (PDR) in 9 patients. Demographic profiles such as age and DM duration were analyzed. The biochemical profiles of the blood were analyzed for glucose, BUN, creatinine, lactate dehydrogenase, total cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and LDL diameter. In addition, various cytokines such as IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) were also analyzed.

Results: DM duration (P<0.001) was longer in DM patients with DR compared to DM patients without DR. Glucose (P=0.001), total cholesterol (P=0.005) and

LDL-C (P=0.025) were significantly higher in DM patients with DR than in DM patients without DR. In trend analysis, the severity of DR showed a significant positive correlation with DM duration (P<0.001), glucose (P<0.001), BUN (P=0.021), creatinine (P=0.004), total cholesterol (P=0.004), LDL-C (P=0.023) and MCP-1 (P=0.049). The independent risk factors associated with the presence of DR by multiple logistic regression analysis were DM duration (OR=1.30; 95% CI, 1.14-1.48 per year), glucose (OR=1.01; 95% CI, 1.00-1.02 per 1 mg/dL), total cholesterol (OR=1.03; 95% CI, 1.01-1.05 per 1 mg/dL) and IL-1β (OR=1.30; 95% CI, 1.14-1.48 per 1 pg/mL). DM duration (P=0.004) was longer than in DM patients with non-proliferative diabetic retinopathy (NPDR). IL-6 (P=0.002) and TNF-α (P=0.012) levels were higher in DM patients with PDR compared to those with NPDR. DM patients with PDR were younger than those with NPDR(P=0.034). And the LDL diameter (P=0.003) was smaller in DM patients with PDR compared to those with NPDR.

Conclusions: DM duration seems to be an important risk factor in the prediction of diabetic retinopathy. The serum glucose, total cholesterol, and LDL-cholesterol levels may be useful in the evaluation for diabetic retinopathy in DM patients. In addition, levels of IL-6 and TNF-α, as well as LDL diameter may also be useful for the prediction of PDR in DM patients with diabetic retinopathy.

Key words: biochemical parameter, cytokine, diabetic retinopathy (DR), low density lipoprotein diameter (LDL diameter), proliferative diabetic retinopathy (PDR)

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I. INTRODUCTION

Diabetic retinopathy (DR) is a common microvascular complication of diabetes mellitus (DM), resulting in blindness in over 10,000 diabetic people per year.[1] If DR goes undetected or is left untreated, it can have an aggravating impact on the patient's quality of life and put a significant burden on health care costs.[2] Accordingly, early diagnosis for DR and proper management can improve the outcome of DM patients.

There are some methods to diagnose DR such as ophthalmoscopy, fluorescein angiography and fundus photography. The Grading of stereoscopic color fundus photographs in seven standard fields (SSFs), as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) group, is a recognized standard for the detection of diabetic retinopathy.[3] However, all these ophthalmic diagnostic approaches must be conducted by a professional ophthalmologist and also require invasive and expensive procedures. Therefore, the development of peripheral blood biochemical parameters for DR could be helpful for early detection and management of DM patients with DR. There have been few studies to identify useful peripheral biochemical parameters of diabetic retinopathy in human peripheral blood.[4, 5]

The aim of this study was to evaluate the clinical usefulness of peripheral blood

biochemical parameters for the prediction and management of DR, various peripheral blood biochemical parameters were compared between DM patients with and without DR.

Ⅱ. MATERIALS AND METHODS

1. Subjects

For this study, 98 patients were enrolled who had been diagnosed with type 1 or type 2 DM at least 6 months prior and who had been continuously receiving insulin therapy at least 3 months prior at the Yonsei University Health System, Seoul, Korea. Of the 98 patients (mean age 58±9.9), there were 48 males (mean age 57.9±9.8) and 50 females (mean age 58.1±10.1). The age of the patients ranged from 30 to 77 years (median age 60).

Sixty patients had DM with DR and 38 were DM without DR. DM patients with DR were classified according to the degree of severity: mild (n=29), moderate (n=11), severe (n=11) and proliferative diabetic retinopathy group (n=9). The diagnosis of DM was made based on 1985 World Health Organization criteria.[6] Exclusion criteria included other eye disorders that could interfere with the diagnosis of DR, acute myocardial infarction, any organ failure, liver disease, stroke, systemic infection and experience of laser photo-coagulation therapy.

In addition, 41 healthy volunteers who had visited Severance Hospital Health Promotion Center for a regular health examination were enrolled as healthy controls, and they all were free of DM, hypercholesterolemia, hypertension and coronary heart disease. The 41 healthy control subjects (mean age 44.8±10.9) included 26 males (mean age 43.9±10.6) and 15 females (mean age 46.3±11.5). This study protocol was approved by the Institutional Review Board (IRB) of the Yonsei University Health System on February 8th 2007. The IRB of Yonsei University Health System is organized and operated according to the ICH-GCP (International Conference on Harmonization-Good Clinical Practice) and the applicable regulations.

2. Diagnosis of diabetic retinopathy

Diabetic retinopathy (DR) was diagnosed by ophthalmoscopy and fluorescein angiography through dilated pupils by an ophthalmologist in the Yonsei University Health System. The patients were classified according to the presence or absence of DR and the degree of severity using the final scale of the Early Treatment Diabetic Retinopathy Study (ETDRS) Classification[3] as follows: 0, absent DR (DM patients without DR); 1, DM patients with mild DR; 2, DM patients with moderate DR; 3, DM patients with severe DR; 4, DM patients with proliferative DR (PDR).

3. Blood sample collection

From March 2006 to January 2007, overnight fasting serum and EDTA plasma samples were taken from the patients and aliquotted and then frozen at -76°C until analysis. Serum samples were thawed at room temperature and used for biochemical assay. EDTA plasma samples were thawed at room temperature and used for the cytokine assay and LDL diameter analysis.

4. Biochemical assay

All parameters described below were analyzed based on a spectrophotometric method using the Hitachi-7600 D-module automatic Analyzer (Hitachi Ltd., Tokyo, Japan) with each commercial reagent. Serum glucose was spectrophotometrically measured using a hexokinase method that measures the NADP formed from hexokinase-catalyzed transformations of glucose and various intermediates with gluco-quant®glucose/HK reagent (Roche Diagnostics, Mannheim, Germany). BUN was measured by urease using the glutamate dehydrogenase (GLDH) method with Wako L-type UN reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Creatinine measurement was based on the Jaffe reaction, in which creatinine was reacted with picrate using Daiichi Clinimate CREA reagent (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). Lactate dehydrogenase (LDH) was measured by the

enzymatic reaction catalyzed by lactate dehydrogenase using a spectrophotometric enzyme assay with Wako L-type LDH reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Total cholesterol, high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were measured using enzymatic colorimetry with the Daiichi reagent (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). Triglyceride was measured using enzymatic colorimetry with the Roche triglycerides GPO-PAP reagent (Roche Diagnostics, Mannheim, Germany).

5. Measurement of LDL diameter

To evaluate the LDL diameter (peak particle diameter), LDL was isolated from the plasma, according to the method described by Griffin et al..[7] LDL was isolated from the plasma at a density of 1.019-1.063 g/mL by density gradient ultracentrifugation. Then, electrophoresis was performed using a pore-gradient lipoprotein system (CBS Scientific, Del Mar, CA) with commercially available, non-denaturing 2-16% polyacrylamide gels (Alamo Gels Inc, San Antonio, TX). After electrophoresis, the gels were fixed for 30 minutes in sulphosalicyclic acid and stained with Coomassie blue for 1 hour. The gels were destained in 7.5% acetic acid for 24 hours and standardized against markers: polystyrene latex beads (36 nm), thyroglobulin (17 nm), apoferritin (12.2 nm) and catalase (10.4 nm). The gels were scanned using a GS-800 Calibrated Imaging Densitometry (Bio-Rad Laboratories, Graz, Austria).

6. Cytokine assay

The biochip array, Evidence investigator™ cytokine and growth factor array (RANDOX Laboratories Ltd., Crumlin, UK) were used to perform simultaneous quantitative detection of multiple analytes from a single patient sample.[8] Evidence investigator™ quantitatively tested for IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α), monocyte chemo -attractant protein-1 (MCP-1) by a sandwich chemiluminescent immunoassay. Increased levels of cytokines in a specimen lead to increased binding of an antibody

labeled with horseradish peroxidase (HRP) and thus an increase in the chemiluminescence being emitted.

The light signal generated from each of the test regions on the biochip was detected using digital imaging technology and compared to that from a stored calibration curve. The concentration of analyte presented in the sample was calculated from the calibration curve. Quality control procedures were all implemented in cytokine profiles which had passed pre-defined acceptance criteria to guarantee a high degree of precision.

7. Statistical analysis

Data were expressed as the mean±SD or median (interquartile range). Comparison between DM patients with and without DR were conducted by independent samples t-test. The association between all parameters and the degree of severity of DR were evaluated: 0, DM patients without DR; 1, DM patients with mild DR; 2, DM patients with moderate DR; 3, DM patients with severe DR; 4, DM patients with proliferative DR (PDR). Independent factors associated with the risk of DR among the biochemical parameters were analyzed by multiple logistic regression analysis. Of the 60 DM patients with DR, comparisons of peripheral biochemical parameters between 51 DM patients with non-proliferative DR (NPDR) and 9 DM patients with proliferative diabetic retinopathy (PDR) were performed by the Mann-Whitney *U* test. Multiple logistic regression analysis were also used to find independent factors associated with the risk of PDR .

SPSS 12.0 (SPSS Inc., Chicago, IL) and Analyze-it (Analyse-it Software Ltd., Leeds, UK) were used for statistical analysis. A *P*-value less than 0.05 (two-tailed) was considered to be statistically significant.

Ⅲ. RESULTS

1. Comparison of biochemical and demographic profiles between diabetes mellitus patients with and without diabetic retinopathy

The DM duration (P<0.001) was longer in patients with DR than in patients without DR. Glucose (P=0.001), total cholesterol (P=0.005) and LDL-C (P=0.025) were higher in DM patients with DR than in DM patients without DR (Fig.1). Other profiles such as BUN, creatinine, LDH, HDL-C and triglyceride did not show a significant difference (P>0.05) between the two groups (Table 1).

2. Comparison of LDL diameter between diabetes mellitus patients with and without diabetic retinopathy

In a comparison of the LDL diameter (peak particle diameter), there was no significant difference between DM patients with (29.96±1.16 nm) and without (26.74±1.05 nm) DR (P>0.05) (Table 1).

3. Comparison of cytokine assays between diabetes mellitus patients with and without diabetic retinopathy

Peripheral blood levels of 12 cytokines such as IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, VEGF, IFN-γ, TNF-α and MCP-1 were estimated and compared between DM patients with and without DR.

However, none of the above cytokines were significantly different between DM patients with and without DR (P>0.05) (Table 1).

Table 1. Comparison of demographic and biochemical profiles between diabetes mellitus patients with and without diabetic retinopathy

Parameters	Normal (n=41)	DM without DR (n=38)	DM with DR (n=60)	*P-value
Age (years)	44.76 ± 10.88	57.53 ± 8.64	58.32 ± 10.67	NS
DM duration (years)	0.00 ± 0.00	8.61 ± 4.93	15.07 ± 6.45	<0.001
Glucose (mg/dL)	86.90 ± 7.39	141.03 ± 48.97	199.18 ± 112.43	0.001
BUN (mg/dL)	13.30 ± 3.32	15.83 ± 4.39	17.18 ± 6.30	NS
Creatinine (mg/dL)	0.92 ± 0.16	0.88 ± 0.20	1.08 ± 0.83	NS
Total cholesterol (mg/dL)	193.66 ± 41.04	165.82 ± 26.46	183.87 ± 32.54	0.005
HDL-cholesterol (mg/dL)	57.41 ± 14.85	52.66 ± 8.51	55.62 ± 13.34	NS
LDL-cholesterol (mg/dL)	128.27 ± 32.99	105.61 ± 24.02	118.02 ± 27.53	0.025
Lactate dehydrogenase (IU/L)	323.24 ± 50.99	359.47 ± 53.22	348.38 ± 73.33	NS
Triglyceride (mg/dL)	167.12 ± 195.57	169.16 ± 125.45	173.60 ± 101.53	NS
LDL diameter (nm)	27.08 ± 1.14	26.74 ± 1.05	26.96 ± 1.16	NS
IL-1α (pg/mL)	0.81 ± 2.45	0.42 ± 0.41	0.54 ± 1.12	NS
IL-1β (pg/mL)	1.60 ± 3.64	0.95 ± 0.99	1.79 ± 5.08	NS
IL-2 (pg/mL)	3.01 ± 8.83	2.88 ± 8.10	3.08 ± 6.69	NS
IL-4 (pg/mL)	4.18 ± 3.55	6.86 ± 20.82	4.40 ± 7.81	NS
IL-6 (pg/mL)	1.26 ± 2.32	5.05 ± 2.78	1.44 ± 1.57	NS
IL-8 (pg/mL)	4.55 ± 7.93	1.45 ± 1.62	7.88 ± 13.39	NS
IL-10 (pg/mL)	0.82 ± 2.36	0.70 ± 0.59	1.11 ± 2.76	NS
EGF (pg/mL)	13.15 ± 39.09	45.28 ± 35.81	42.22 ± 38.97	NS
VEGF (pg/mL)	26.20 ± 21.40	78.12 ± 67.77	61.44 ± 49.14	NS
IFN-γ (pg/mL)	1.26 ± 3.02	1.61 ± 1.70	1.74 ± 3.34	NS
TNF-α (pg/mL)	3.41 ± 2.99	3.78 ± 3.51	4.13 ± 3.74	NS
MCP-1 (pg/mL)	87.05 ± 77.70	145.16 ± 57.26	163.57 ± 122.26	NS

*P-value was represented by independent two-samples t-test between DM patients with DR and DM patients without DR. Abbreviations: DM, diabetes mellitus; DR, diabetic retinopathy; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor ; INF-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; NS, non-significant.

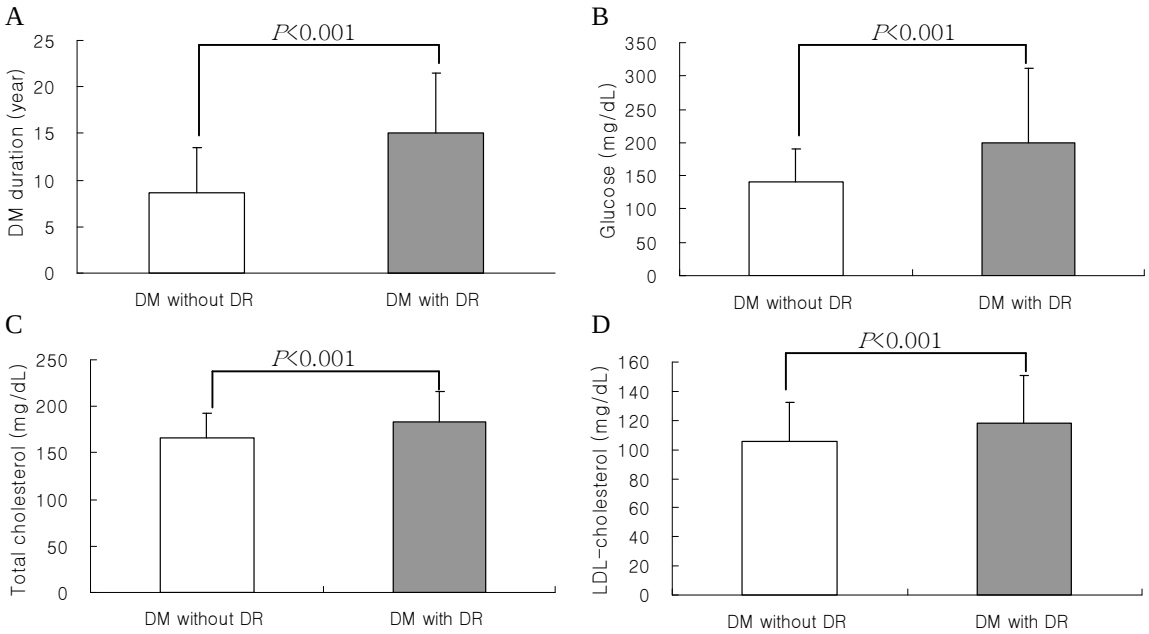

Fig.1. Comparison of (A) diabetes mellitus duration, peripheral blood level of (B) glucose, (C) total cholesterol and (D) LDL-cholesterol between DM patients without diabetic retinopathy and with diabetic retinopathy. Bars indicated mean±SD. All of above comparative parameters showed statistically significant difference (P<0.05).

Abbreviations: DM, diabetes mellitus; DR, diabetic retinopathy; LDL-C, low density lipoprotein-cholesterol.

4. Clinical and biochemical characteristics according to the degree of severity of diabetic retinopathy

When the demographics and biochemical parameters were analyzed according to the degree of severity of DR, some parameters showed statistically significant trends.

In the demographic and medical history, the DM duration showed a positive association with increasing severity of DR ($P<0.001$).

In the biochemical profiles, glucose ($P<0.001$), BUN ($P=0.021$) and creatinine ($P=0.004$) showed a positive association with increasing severity of DR and in the lipid profiles, total cholesterol ($P=0.004$) and LDL-C ($P=0.023$) showed a positive association with increasing severity of DR.

In the cytokine assays, only MCP-1 showed a significant positive association with increasing severity of DR ($P=0.049$) (Table 2).

Table 2. Characteristics of peripheral blood biochemical parameters in relation to the degree of severity of diabetic retinopathy

Parameters	DM without DR (n=38)	DM with mild DR (n=29)	DM with moderate DR (n=11)	DM with severe DR (n=11)	DM with PDR (n=9)	*P for trend
Age (years)	57.53 ± 8.64	58.79 ± 10.65	58.55 ± 9.76	62.55 ± 8.30	51.33 ± 12.53	NS
DM duration (years)	8.61 ± 4.93	13.59 ± 5.68	12.82 ± 5.64	16.82 ± 7.53	20.44 ± 5.64	<0.001
Glucose (mg/dL)	141.03 ± 48.97	176.24 ± 88.58	200.27 ± 100.14	213.00 ± 141.24	254.89 ± 150.44	<0.001
BUN (mg/dL)	15.83 ± 4.39	16.03 ± 3.77	17.05 ± 4.46	16.95 ± 5.08	21.34 ± 12.70	0.021
Creatinine (mg/dL)	0.88 ± 0.20	0.93 ± 0.20	1.06 ± 0.51	0.95 ± 0.24	1.77 ± 1.98	0.004
Total cholesterol (mg/dL)	165.82 ± 26.46	181.34 ± 29.60	174.27 ± 33.61	193.18 ± 42.52	192.33 ± 26.82	0.004
HDL-cholesterol (mg/dL)	52.66 ± 8.51	55.97 ± 15.32	56.09 ± 6.69	58.73 ± 14.67	50.11 ± 10.89	NS
LDL-cholesterol (mg/dL)	105.61 ± 24.02	117.17 ± 25.99	107.27 ± 29.83	127.27 ± 31.83	122.56 ± 23.30	0.023
Lactate dehydrogenase (IU/L)	359.47 ± 53.22	334.76 ± 59.02	328.09 ± 68.39	362.55 ± 58.69	399.78 ± 113.28	NS
Triglyceride (mg/dL)	169.16 ± 125.45	158.66 ± 75.53	188.91 ± 105.86	155.82 ± 131.00	224.78 ± 126.81	NS
LDL diameter (nm)	26.74 ± 1.05	27.04 ± 1.06	26.69 ± 1.09	27.83 ± 1.01	25.97 ± 1.00	NS
IL-1α (pg/mL)	0.42 ± 0.41	0.46 ± 0.61	1.26 ± 2.34	0.19 ± 0.27	0.34 ± 0.28	NS
IL-1β (pg/mL)	0.95 ± 0.99	1.28 ± 2.02	5.23 ± 11.12	0.56 ± 0.45	0.71 ± 0.64	NS
IL-2 (pg/mL)	2.88 ± 8.10	2.54 ± 5.47	5.90 ± 12.56	2.84 ± 2.72	1.71 ± 2.09	NS
IL-4 (pg/mL)	6.86 ± 20.82	3.34 ± 1.30	9.67 ± 17.79	3.28 ± 0.66	2.79 ± 0.73	NS
IL-6 (pg/mL)	1.45 ± 1.62	1.15 ± 0.92	1.36 ± 1.78	0.81 ± 0.33	3.24 ± 2.56	NS
IL-8 (pg/mL)	5.05 ± 2.78	8.23 ± 17.42	12.28 ± 12.31	4.29 ± 1.70	5.79 ± 4.05	NS
IL-10 (pg/mL)	0.70 ± 0.59	0.66 ± 0.85	1.57 ± 2.72	0.45 ± 0.50	2.82 ± 6.27	NS
EGF (pg/mL)	45.28 ± 35.81	44.48 ± 42.59	42.82 ± 37.84	29.85 ± 27.07	49.36 ± 43.09	NS
VEGF (pg/mL)	78.12 ± 67.77	53.20 ± 37.01	56.23 ± 39.83	68.79 ± 49.75	85.35 ± 83.28	NS
IFN-γ (pg/mL)	1.61 ± 1.70	1.17 ± 2.40	2.85 ± 6.35	1.96 ± 2.12	1.95 ± 1.79	NS
TNF-α (pg/mL)	3.78 ± 3.51	3.80 ± 3.18	4.57 ± 5.97	3.29 ± 0.58	5.68 ± 4.34	NS
MCP-1 (pg/mL)	145.16 ± 57.26	140.27 ± 54.47	136.47 ± 28.02	242.79 ± 255.70	174.92 ± 73.42	0.049

*For each variable, the linear trend with the severity of DR was tested. *P* for trend less than 0.05 were considered statistically significant.

Abbreviations: DM, diabetes mellitus; DR, diabetic retinopathy; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; NS, non-significant.

5. Risk factors independently associated with the presence of diabetic retinopathy in diabetes mellitus patients

The risk factors independently associated with the presence of DR in DM patients were analyzed by using a multiple logistic regression procedure. Among the demographic and biochemical parameters, DM duration (OR=1.30; 95% CI, 1.14-1.48 per year), glucose (OR=1.01; 95% CI, 1.00-1.02 per 1 mg/dL), total cholesterol (OR=1.03; 95% CI, 1.01-1.05 per 1 mg/dL) and IL-1β (OR=1.92; 95% CI, 1.16-3.18 per 1 pg/mL) were identified as possible risk factors of DR (Table3).

6. Comparison of demographics and biochemical parameters between non-proliferative diabetic retinopathy and proliferative diabetic retinopathy

In DM patients with PDR, DM duration (P=0.004) was longer and IL-6 (P=0.002) and TNF-α (P=0.012) levels were higher, compared with DM patients with NPDR. In addition, in DM patients with PDR, the age (P=0.034) was younger and the LDL diameter (P=0.003) was smaller, compared with DM patients with NPDR. Using a multiple logistic regression procedure, any risk factors independently associated with the presence of PDR in DM patients could not be identified (Table 4).

Table 3. Risk factors independently associated with the presence of diabetic retinopathy in diabetes mellitus patients

Risk factor	OR (95% CI)	*P -value
DM duration (per year)	1.30 (1.14-1.48)	<0.001
Glucose (per 1 mg/dL)	1.01 (1.00-1.02)	0.009
Total cholesterol (per 1 mg/dL)	1.03 (1.01-1.05)	0.010
IL-1β (per 1 pg/mL)	1.92 (1.16-3.18)	0.011

*Backward stepwise logistic regression model was used for selection started with all variables and deleted one at a time, in the order they are worst criteria. Variables included in the multivariate model were gender, age, DM duration, glucose, BUN, creatinine, lactate dehydrogenase, total cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), LDL diameter, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1).

Abbreviations: OR, odds ratio; CI, confidence interval.

Table 4. Comparison of demographics and biochemical parameters between diabetes mellitus in non-proliferative diabetic retinopathy and diabetes mellitus in proliferative diabetic retinopathy

Parameters	DM with NPDR (n=51)			DM with PDR (n=9)			*P-value
	mean ± SD	median	interquartile range	mean ± SD	median	interquartile range	
Age (years)	59.55 ± 9.94	61.00	(54.00 - 66.00)	51.33 ± 12.53	51.00	(42.50 - 58.00)	0.034
DM duration (years)	14.12 ± 6.16	13.00	(10.00 - 17.00)	20.44 ± 5.64	20.00	(17.00 - 24.00)	0.004
Glucose (mg/dL)	189.35 ± 103.14	172.00	(118.00 - 265.00)	254.89 ± 150.44	210.00	(140.50 - 309.50)	NS
BUN (mg/dL)	16.45 ± 4.16	15.60	(12.80 - 19.20)	21.34 ± 12.70	18.50	(15.85 - 21.15)	NS
Creatinine (mg/dL)	0.96 ± 0.30	0.90	(0.80 - 1.10)	1.77 ± 1.98	1.30	(0.85 - 1.40)	NS
Total cholesterol (mg/dL)	182.37 ± 33.45	179.00	(161.00 - 205.00)	192.33 ± 26.82	192.00	(175.50 - 209.00)	NS
HDL-cholesterol (mg/dL)	56.59 ± 13.59	53.00	(47.00 - 65.00)	50.11 ± 10.89	50.00	(41.00 - 56.50)	NS
LDL-cholesterol (mg/dL)	117.22 ± 28.34	119.00	(103.00 - 129.00)	122.56 ± 23.30	125.00	(101.50 - 141.50)	NS
Lactate dehydrogenase (IU/L)	339.31 ± 61.10	339.00	(293.00 - 380.00)	399.78 ± 113.28	372.00	(319.50 - 433.50)	NS
Triglyceride (mg/dL)	164.57 ± 95.05	140.00	(102.00 - 207.00)	224.78 ± 126.81	163.00	(137.00 - 355.00)	NS
LDL diameter (nm)	27.13 ± 1.11	27.10	(26.20 - 27.90)	25.97 ± 1.00	25.60	(25.35 - 26.75)	0.003
IL-1α (pg/mL)	0.57 ± 1.21	0.40	(0.00 - 0.54)	0.34 ± 0.28	0.42	(0.00 - 0.48)	NS
IL-1β (pg/mL)	1.98 ± 5.49	0.80	(0.00 - 0.98)	0.71 ± 0.64	0.73	(0.00 - 1.22)	NS
IL-2 (pg/mL)	3.33 ± 7.19	0.00	(0.00 - 3.58)	1.71 ± 2.09	0.00	(0.00 - 3.52)	NS
IL-4 (pg/mL)	4.69 ± 8.44	3.09	(2.65 - 3.81)	2.79 ± 0.73	2.51	(2.36 - 3.30)	NS
IL-6 (pg/mL)	1.12 ± 1.08	0.82	(0.62 - 1.09)	3.24 ± 2.56	2.46	(1.06 - 5.50)	0.002
IL-8 (pg/mL)	8.25 ± 14.42	4.10	(3.09 - 7.61)	5.79 ± 4.05	4.19	(2.59 - 8.97)	NS
IL-10 (pg/mL)	0.81 ± 1.45	0.58	(0.00 - 0.68)	2.82 ± 6.27	0.98	(0.54 - 1.10)	NS
EGF (pg/mL)	40.97 ± 38.52	29.71	(14.15 - 59.65)	49.36 ± 43.09	40.25	(18.70 - 66.46)	NS
VEGF (pg/mL)	57.22 ± 40.23	52.21	(24.87 - 71.77)	85.35 ± 83.28	59.32	(37.74 - 112.30)	NS
IFN-γ (pg/mL)	1.70 ± 3.56	0.00	(0.00 - 1.86)	1.95 ± 1.79	1.62	(0.81 - 2.47)	NS
TNF-α (pg/mL)	3.86 ± 3.61	3.17	(2.79 - 3.81)	5.68 ± 4.34	3.68	(3.43 - 6.10)	0.012
MCP-1 (pg/mL)	161.56 ± 129.41	141.00	(118.42 - 167.34)	174.92 ± 73.42	164.96	(118.76 - 217.14)	NS

*P-value was represented by Mann-Whitney U test between DM patients with non-proliferative diabetic retinopathy (NPDR) and DM patients with proliferative diabetic retinopathy (PDR).

Abbreviations: DM, diabetes mellitus; DR, diabetic retinopathy; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; NS, non-significant.

Ⅳ. DISCUSSION

In this study, the levels of various biochemical parameters were measured in peripheral blood and were compared between DM patients without and with DR.

There have been several studies measuring elevated inflammatory proteins, cytokines or adhesion molecules in the vitreous fluid or plasma of DM patients with DR.[9-13] Huang et al.[14] reported that peripheral blood level of homocystein of DM patients with DR was higher than that of DM patients without DR, and other profiles such as glucose, total glucose and LDL-cholesterol (LDL-C) were not significantly different. However, the results of our study showed the duration of DM in patients with DR was longer than that in patients without DR. In addition, the peripheral blood levels of glucose, total cholesterol and LDL-C in DM patients with DR were higher than those in DM patients without DR ($P<0.05$). The discrepancy between the results of this study and those of Huang et al. might also be due to the difference in sample size and the types of DM studied, because our study included several type 1 DM patients.

In recent studies, dyslipidemia was reported to be a risk factor for diabetic renal disease, but the effect of serum lipids on DR and macular edema is still under investigation and there have been several studies with conflicting results for lipid profiles in DR patients.[15-17]

The EURODIAB IDDM complications study group[18] has reported that cholesterol levels are related to all levels of retinopathy and triglycerides are associated with moderately severe non-proliferative DR and proliferative DR. Nevertheless, in our study, triglyceride did not show a noteworthy association with the presence of DR or PDR.

The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC)[19] reported that the LDL diameter is negatively associated with DR of type 1 DM in males. In addition, in our study, the LDL diameter (peak particle diameter) was only smaller in DM patients with PDR than NPDR, but was not associated with the presence of DR. The different result between our study and DCCT/EDIC study might be caused by subject's character such as DM type difference, because most patients of our study had type

2 DM and few had type 1 DM. The lipid profile and LDL diameter were not decisive risk factors for DR, but could be screened for in routine laboratory tests for DR in DM patients.

The degree of severity should be evaluated to decide the management of DM patients. In our results, DM duration, glucose, BUN, creatinine, total cholesterol, LDL-C and MCP-1 showed linear trend as DR severity increased (P <0.05). These linear trends do not indicate an exact proportional association between any of these markers and the degree of DR severity, but rather, the trends may imply some propensity with significant linearity.

The independent risk factors associated with presence of DR or PDR were also analyzed in our study. Varma R et al.[20] reported biological risk factors were associated with DR in Latinos with type 2 diabetes mellitus (T2DM), and one of the factors independently associated with a greater risk of having any DR was a longer duration of DM (per year, OR=1.08, P<0.0001). In this study, DM duration (OR=1.30; 95% CI, 1.14-1.48 per year) was also independently associated with the presence of DR. In addition to DM duration, glucose, total cholesterol and IL-1β levels were also independent risk factors associated with the presence of DR.

The proinflammatory cytokine, IL-1β, is known to induce vascular dysfunction and cell death. Doganay S et al.[21] reported the serum levels of IL-1β was below the detection limits of the assay (<5.0 pg/mL) in all DM patients with or without DR. Patel JI et al.[22] suggested the concentration of vitreous IL-1β in the non-proliferative diabetic retinopathy (NPDR) patients was below the sensitivity of the assay, but the mean concentration in PDR patients was higher. Demircan N et al.[23] reported that increased vitreous IL-1β levels may play a significant role in the pathogenesis of PDR.

Nevertheless, in our study, IL-1β was an independent risk factor only for the presence of DR, but not PDR. Therefore, a more prospective study with a larger study population is necessary to clarify the association between IL-1β levels and DR.

The last concerning results were from the comparison of biochemical parameters between NPDR and PDR patients. The DM duration was longer in DM patients with PDR than in those with NPDR. Further, IL-6 and TNF-α levels were higher, but patient age was younger and the LDL diameter was smaller in DM patients with PDR

compared to those with NPDR. Doganay S et al.[21] reported that serum IL-6 levels were below the detection limits of the assay (<5.0 pg/ml) in both NPDR or PDR patients. Mocan MC et al.[24] reported that significantly higher intra-vitreal IL-6 concentrations were found in patients with PDR, compared with control subjects.

Funatsu H et al.[25] reported aqueous levels of IL-6 were significantly correlated with the severity of diabetic retinopathy. Therefore, the vitreous level of IL-6 in PDR patients must be important in pathogenesis of PDR, but the serum level of IL-6 needs further study. Our results also showed that the plasma level of IL-6 was higher in PDR patients than in NPDR patients.

Demircan N et al.[23] also reported that vitreous TNF-α levels may play a significant role in the pathogenesis of PDR. Doganay S et al.[21] also reported the serum TNF-α level is higher in PDR patients like our study. Hence, peripheral level of IL-6 and TNF-α might be useful biochemical parameters for PDR.

In other studies, many cytokines and growth factors, including basic fibroblast growth factor (bFGF)[13], insulin-like growth factor (IGF)[26] or vascular endothelial growth factor (VEGF)[27, 28] could be play an important role in pathogenesis of DR or PDR. VEGF is a potent activator of angiogenesis, enhances collateral vessel formation and increases the permeability of the microvasculature. In a plasma VEGF study[29], patients with PDR had significantly raised plasma VEGF when compared with control groups (P = 0.001). However, there have been some reports[25, 30] that vitreous levels of VEGF are not influenced by its serum concentration in diabetic retinopathy. In addition, Funatsu H et al.[25] reported there was no significant correlation between aqueous and plasma levels of VEGF, but VEGF levels were much higher in the aqueous than in the plasma.

The peripheral levels of VEGF were higher in DR or PDR group than in healthy control group, but not significantly different between NDR and DR or between NPDR and PDR patients in our study.

In recent case-control studies, several polymorphisms at the VEGF 5'-regulatory region have been characterized and evaluated as risk alleles for the susceptibility or progression of DR.[31, 32] This might imply that not only the level of VEGF but also VEGF polymorphisms can contribute to DR or PDR progression.

There were some limitations such as the relatively small scale (n=60) and numerical

discordance between NPDR (n=51) with PDR (n=9) cases. Prospective studies of larger study populations are necessary to clarify various suggestive peripheral blood biochemical parameters of DR or PDR, such as lipid profiles, LDL diameter, cytokines such as IL-1β, IL-6, TNF-α and VEGF.

V. CONCLUSION

In this study, the peripheral blood biochemical parameters in diabetic retinopathy (DR) were analyzed and some meaningful results were obtained. Diabetes mellitus (DM) duration was longer in DM patients with DR than in those without DR. In addition, the levels of serum glucose, total cholesterol and LDL-cholesterol were higher in DM patients with DR than in DM patients without DR. And DM duration, glucose, total cholesterol and IL-1β were identified as independent risk factors associated with the presence DR.

In DM patients with PDR, the DM duration was longer, patients were younger, IL-6 and TNF-α levels were higher and the LDL diameter was smaller than in DM patients with NPDR.

Therefore, monitoring of serum glucose, total cholesterol and LDL-cholesterol may be useful for evaluating DR in DM patients. IL-6, TNF-α, and LDL diameter may also be useful for predicting PDR in DR patients.

Ⅵ. REFERENCES

1. Fong DS, Aiello LP, Ferris FL 3rd, Klein R. Diabetic retinopathy. Diabetes Care 2004;27:2540-53.
2. Girach A, Manner D, Porta M. Diabetic microvascular complications: can patients at risk be identified? A review. Int J ClinPract 2006;60:1471-83.
3. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs–an extension of the modified Airlie House classification: ETDRS report number 10. Ophthalmology 1991;98:786-806.
4. Meleth AD, Agron E, Chan CC, Reed GF, Arora K, Byrnes G, et al. Serum inflammatory markers in diabetic retinopathy. Invest Ophthalmol Vis Sci 2005;46:4295-301.
5. Canton A, Burgos R, Hernandez C, Mateo C, Segura RM, Mesa J, et al. Hepatocyte growth factor in vitreous and serum from patients with proliferative diabetic retinopathy. Br J Ophthalmol 2000;84:732-5.
6. WHO Study Group. Diabetes mellitus. Report of a WHO study group. World Health Organ Tech Rep Ser 1985;727:1-113.
7. Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. Atherosclerosis 1990;83:159-67.
8. Molloy RM, Mc Connell RI, Lamont JV, FitzGerald SP. Automation of biochip array technology for quality results. Clin Chem Lab Med 2005;43:1303-13.
9. Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Macrophage migration inhibitory factor levels in the vitreous of patients with proliferative diabetic retinopathy. Br J Ophthalmol 2000; 84:636-9.
10. Malik RA, Li C, Aziz W, Olson JA, Vohra A, McHardy KC, et al. Elevated plasma CD105 and vitreous VEGF levels in diabetic retinopathy. J Cell Mol Med 2005;9:692-7.
11. Jacqueminet S, Ben Abdesselam O, Chapman MJ, Nicolay N, Foglietti MJ, Grimaldi A, et al. Elevated circulating levels of matrix metalloproteinase-9 in type 1 diabetic patients with and without retinopathy. Clin Chim Acta

2006;367:103-7.

12. Shinoda K, Ishida S, Kawashima S, Wakabayashi T, Matsuzaki T, Takayama M, et al. Comparison of the levels of hepatocyte growth factor and vascular endothelial growth factor in aqueous fluid and serum with grades of retinopathy in patients with diabetes mellitus. Br J Ophthalmol 1999;83:834-7.
13. Sivalingam A, Kenney J, Brown GC, Benson WE, Donoso L. Basic fibroblast growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch Ophthalmol 1990;108:869-72.
14. Huang EJ, Kuo WW, Chen YJ, Chen TH, Chang MH, Lu MC, et al. Homocysteine and other biochemical parameters in Type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy. Clin Chim Acta 2006;366:293-8.
15. Chowdhury TA, Hopkins D, Dodson PM, Vafidis GC. The role of serum lipids in exudative diabetic maculopathy: is there a place for lipid lowering therapy? Eye 2002;16:689-93.
16. Sinav S, Onelge MA, Onelge S, Sinav B. Plasma lipids and lipoproteins in retinopathy of type I (insulin-dependent) diabetic patients. Ann Ophthalmology 1993;25:64-6.
17. Marshall G, Garg SK, Jackson WE, Holmes DL, Chase HP. Factors influencing the onset and progression of diabetic retinopathy in subjects with insulin-dependent diabetes mellitus. Ophthalmology 1993;100:1133-9.
18. Sjolie AK, Stephenson J, Aldington S, Kohner E, Janka H, Stevens L, et al. Retinopathy and vision loss in insulin-dependent diabetes in Europe. The EURODIAB IDDM Complications Study. Ophthalmology 1997;104:252-60.
19. Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, et al. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci 2004;45:910-8.
20. Varma R, Macias GL, Torres M, Klein R, Pena FY, Azen SP, et al. Biologic risk factors associated with diabetic retinopathy The Los Angeles Latino Eye Study. Ophthalmology (in press).
21. Doganay S, Evereklioglu C, Er H, Turkoz Y, Sevinc A, Mehmet N, et al. Comparison of serum NO, TNF-alpha, IL-1beta, sIL-2R, IL-6 and IL-8 levels

with grades of retinopathy in patients with diabetes mellitus. Eye 2002;16:163-70.

22. Patel JI, Saleh GM, Hykin PG, Gregor ZJ, Cree IA. Concentration of haemodynamic and inflammatory related cytokines in diabetic retinopathy. Eye (in press).
23. Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye 2006;20:1366-9.
24. Mocan MC, Kadayifcilar S, Eldem B. Elevated intravitreal interleukin-6 levels in patients with proliferative diabetic retinopathy. Can J Ophthalmol 2006;41:747-52.
25. Funatsu H, Yamashita H, Shimizu E, Kojima R, Hori S. Relationship between vascular endothelial growth factor and interleukin-6 in diabetic retinopathy. Retina 2001;21:469-77.
26. Grant M, Russel B, Fitzgerald C, Merimee TJ. Insulin-like growth factors in vitreous: studies in control and diabetic subjects with neovascularization. Diabetes 1986;35:416-20.
27. Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 1994;118:445-50.
28. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480-7.
29. Lip PL, Belgore F, Blann AD, Hope-Ross MW, Gibson JM, Lip GY, et al. Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. Invest Ophthalmol Vis Sci 2000;41:2115-9.
30. de Vries HE, Blom-Roosemalen MC, van Oosten M, de Boer AG, van Berkel TJ, Breimer DD, et al. The influence of cytokines on the integrity of the blood-brain barrier in vitro. J Neuroimmunol 1996;64:37-43.
31. Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, et al. A common polymorphism in the 5'-untranslated region of the VEGF gene is

associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51:1635-9.

32. Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 2004;53:861-4.

ABSTRACT (IN KOREAN)

당뇨환자에서 당뇨망막병증 유무에 따른 말초혈액 생화학 표지자들의 비교 분석

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서론: 당뇨망막병증을 예측하는 말초혈액 생화학 표지자들의 임상적인 유용성을 평가하기 위하여, 당뇨 환자들을 대상으로 여러 종류의 생화학 표지자들의 혈청 혹은 혈장 농도를 측정하여 비교 평가하였다.

방법; 총 98명의 당뇨 환자와 41명의 건강 성인을 대상으로 말초혈액의 생화학 표지자를 분석하였다. 당뇨환자군은 당뇨망막병증이 없는 38명과 당뇨망막병증이 있는 60명으로 구성되었으며, 당뇨망막병증 환자군은 다시 비증식성당뇨망막병증 51명과 증식성당뇨망막병증 9명으로 구성되었다. 나이와 당뇨 유병기간 및 혈중 glucose, BUN, creatinine, lactate dehydrogenase, total cholesterol, HDL-C (high density lipoprotein-cholesterol), LDL-C (low density lipoprotein-cholesterol), LDL 직경, triglyceride 그리고 싸이토카인 즉, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), interferon-gamma (INF-ɣ), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1)을 분석하였다.

결과: 당뇨 유병기간 (P<0.001)은 당뇨망막병증이 있는 당뇨환자군에서 당뇨망막병증이 없는 당뇨환자군보다 유의하게 길었으며, glucose (P=0.001), total cholesterol (P=0.005) 그리고 LDL-C (P=0.025)은 보다 높았다. 경향성 분석에서 당뇨망막병증이 심할수록, 당뇨의 유병기간 (P for trend <0.001)은 길었으며, glucose (P for trend <0.001), BUN (P for

trend=0.021), creatinine (*P* for trend =0.004), total cholesterol (*P* for trend=0.004), LDL-C (*P* for trend=0.023) 그리고 MCP-1 (*P* for trend=0.049)의 농도는 유의하게 상승하였다. 다중 로지스틱 회귀 분석시 당뇨망막병증의 유병에 관한 유의한 상대위험도를 보이는 항목은 당뇨 유병기간 (OR=1.30; 95% CI, 1.14-1.48 per year), glucose (OR=1.01; 95% CI, 1.00-1.02 per 1 mg/dL), total cholesterol (OR=1.03; 95% CI, 1.01-1.05 per 1 mg/dL) 그리고 IL-1β (OR=1.92; 95% CI, 1.16-3.18 per 1 pg/mL)이었다. 증식성당뇨망막병증 환자에서는 비증식성 당뇨망막병증 환자보다 당뇨 유병기간 (*P*=0.004)은 유의하게 더 길었고, IL-6 (*P*=0.002), TNF-α (*P*=0.012) 혈중 농도는 더 높았으며, 나이 (*P*=0.034)는 보다 더 적었고 LDL 직경 (*P*=0.003)은 더 짧았다.

결론: 당뇨 유병기간은 당뇨환자에서 당뇨망막병증의 발병을 예측하는 중요한 위험인자로 판단되었으며 혈중 glucose, total cholesterol, 그리고 LDL-cholesterol 농도는 당뇨 환자에서 당뇨망막병증을 평가하는데 도움이 되며, IL-6, TNF-α 그리고 LDL 직경은 당뇨망막병증 환자에서 증식성당뇨망막병증을 예측하는데 또한 유용할 것으로 판단되었다.

핵심되는 말: 생화학 표지자, 싸이토카인, 당뇨망막병증, LDL 직경, 증식성당뇨망막병증