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高脂血症病患血管通透性之研究

THE STUDY OF VASCULAR PERMEABILITY CHANGE IN HYPERLIPIDEMIC PATIENTS

一、中文摘要

背景：過去有關人類血管壁通透性的研究極少，至今仍無任何一種方法可在臨床上使用於測量血管的通透性．我們過去的研究已証實眼部螢光測定可用於測量高脂血兔子的血管內皮功能之完整性．而且血管通透性與血中膽固醇濃度有關，且在動脈粥狀硬化的早期便已發生變化．

目的：本研究乃利用眼-水屏障的測量（F60）來評估人類血管通透性的決定因子．

方法與結果：五十八位男性及 74 位女性高脂血症病人(平均年齡：53.6 土 10.6 歲）接受危險因子，理學及生化學檢查及眼部前房螢光光度計測量檢查（F60 值），結果發現 F60 值在停經前婦女最低，男性較高，而停經後婦女最高．F60 值在老年人，糖尿病患者及具血管硬化病變者較高，但在血中高密度膽固醇值（HDL）大於 60 mg/dl 者較低，另外，F60 值與病人的年齡，危險因子的數目，血中膽固醇值，LDL 值，T-CHO/HDL 比值，LDL/HDL 比值呈負相關．多變異分析發現僅 LDL/HDL 比值，年齡，糖尿病及血中膽固醇濃度為 F60 值的獨立決定因子．

結論：眼部前房螢光光度計測量法可非侵襲地評估人類血管的通透性．而決定血管通透性的獨立因子包括病人的血脂濃度，年齡及有無糖尿病的病史等．

關健詞：高脂血症，血管通透性，螢光光度計，血-水屏障．

ABSTRACT

Background. The transfer process in human arterial wall has been studied by only a few investigators. Currently, there is still no widely accepted and clinically noninvasive method to measure the systemic vascular permeability. In our previous study, we used fluorophotometry to evaluate the vascular endothelial integrity in vivo in hyperlipidemic rabbits. The vascular permeability change correlated well with the serum cholesterol level and occurred in the very early

stage of atherosclerosis.
Purpose. To evaluate the status of blood-aqueous barrier (F60) and to define the significant determinants of vascular permeability in human beings.
Methods and Results. Fifty-eight males and 74 female patients (aged 53.6±10.6 years) received risk stratification, physical and blood chemistry examinatios. Fluorophotometry examination was also performed in these hyperlipidemic patients. The F60 value was the lowest in the premenopausal female, higher in male, and the highest in postmenopausal females. It was higher in the aged patients, those with diabetes mellitus, or those with vascular diseases. However, the F60 value was lower in the patients with HDL ≧ 60 mg/dl. The F60 value also correlated positively with the patients' age, number of risk factors, cholesterol , LDL, T-CHO/HDL, LDL/HDL, glucose, and HbA1c levels, and correlated negatively with the HDL level. By multivariate stepwise linear regression analysis, the vascular permeability value correlated significantly with the LDL/HDL ratio, age, diabetes mellitus, and cholesterol level. Other risk factors or the serum TG level were not significant independent predictors for the F60 value.
Conclusion. The vascular permeability could be evaluated noninvasively by fluorophotometry. It was independently determined by the patients' lipid profile, age, and the existence of diabetes mellitus in hyperlipidemic patients.

Key Words: Hyperlipidemia, Vascular Permeability, Fluorophotometry, Blood-aqueous Barrier.

BACKGROUND

Besides smooth muscle cell proliferation and accumulation of connective tissue matrix, changes in endothelial permeability is one of the major common pathogenic mechanisms in atherosclerosis.[1,2] Previously, the arterial transfer process in humans was studied by only a few investigators, who administered labeled albumin or cholesterol orally or intravenously to patients. Arterial specimens were obtained minutes or months later when the patients died or underwent arterial surgery.[3,4,5,6,7] There is still no noninvasive method to measure the vascular permeability at the present time. Because there was a high correlation between the severity of iridic and aortic atherosclerosis as well as vascular wall contents of cholesterol in animal experimental model,[8] changes of the iridic vessels in the eye would be a good indicator of the general status of the vascular system.

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The blood-aqueous barrier could be evaluated clinically by the anterior segment fluorescein angiography,[9,10,11] cell-flare meter,[12] and fluorophotometry.[13,14] Among these, fluorophotometry is the most sensitive method to quantify the breakdown of the blood-aqueous barrier.[15] Taking the advantage of the corneal clarity, the iris vasculature is one of the most easily assessed vessels all-over the body. In our previous study,[16] we used fluorophotometry to evaluate the vascular endothelial integrity in vivo in hyperlipidemic rabbits. The vascular permeability change correlated well with the serum cholesterol level and occurred in the very early stage of atherosclerosis. Moreover, this alteration of vascular permeability preceded the visual evidence of atheromatous plaque.

Hypercholesterolemia is closely related to atherosclerosis and is one of the most important coronary risk factors.[17] In this study, we used fluorophotometry to detect the status of blood-aqueous barrier and intended to define the significant determinants of vascular permeability in hyperlipidemic patients.

MATERIALS AND METHODS

Patients and Study Protocol This study population consisted of 132 patients referred to our lipid clinics for educational consultation or lipid control (with either total cholesterol (T-CHO) or triglyceride (TG) level ≧ 200 mg/dl). The exclusion criteria were the patients with immunologic disease, infectious disease, malignancy, end-stage renal disease, intraocular diseases or operation, history of fluorescein allergy, or poor cooperation. After being fully informed, complete history (coronary artery disease, stroke, peripheral vascular disease, age, sex, hypertension, smoking, family history, diabetes mellitus, personality, physical activity, and medication, etc.) were taken thoroughly. Vascular diseases was defined by typical history, stress tests, duplex sonography, or angiography.

Biochemical measurements Before any life-style modification or medical treatment, blood was sampled preprandially twice within 7 days of fluorophotometric examination. Serum total cholesterol (CHO), triglyceride (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), glucose, hemoglobin A1c, and uric acid levels were determined by automated enzymatic methods.[18,19] The blood levels at different times were averaged for analysis.

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Fluorophotometry Anterior segment fluorophotometry was performed according to a protocol modified from that suggested by Miyake[20] and Fernley.[21] Fluorophotometry was performed using the Fluorotron Master II (Coherent Co.) fitted with an optical anterior segment adapter. After measurement of the lens and corneal autofluorescence, each patient received an intravenous injection of 10% fluorescein sodium (7 mg/kg of body weight). The autofluorescence of the cornea and lens was measured first for background subtraction. Anterior chamber fluorescein concentration was measured 60 minutes after intravenous injection of sodium fluorescein. The mean values of the anterior chamber fluorescence along the visual axis over a 2.0 mm band positioned in the anterior chamber were averaged (F60). We used the F60 to represent the status of the blood-aqueous barrier. As it was impossible to distinguish between the various metabolites of fluorescein using the Fluorotron Master, all fluorophotometric results were expressed as total fluorescence in terms of equivalent concentrations of fluorescein sodium. Data from the 2 eyes were averaged for analysis. The patients were excluded also if the difference of F60 between the 2 eyes was more than 10%.

Statistical Analysis Mean values$\pm$SD were calculated for continuous variables, and absolute and relative frequencies were measured for discrete variables. Between-group comparisons of F60, biochemistry and lipid profiles, and clinical data were analyzed using one-way ANOVA for continuous variables, followed by the Scheffe's test for multiple comparison if the result of ANOVA was significant ($p<0.05$) or by the chi-square test for categorical variables.

The F60 value in the patients with or without various risk or protective factors and atherosclerotic vascular disease was analyzed by the unpaired t test. The correlation of the lipid profiles and the nonlipid variables with respect to the F60 value was carried out with simple linear regression analysis.

The multivariate analysis was performed as stepwise multiple linear regression analysis. The dependent variable was the F60 value. The independent variables included variable risk or protective factors, biochemistry and lipid profiles, and the clinical data, which were significantly or deduced to be associated with the vascular permeability in the univariate analysis. The criteria for inclusion and exclusion of a variable were $p<0.05$ and $p\geq0.10$ in the F-test, respectively.

RESULTS

Patient Characteristics A total of 132 patients were enrolled in this study. There were 58

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males and 74 females. They were aged 53.6±10.6 years (range: 33-78). The clinical characteristics of the patients are summarized in Table 1 according to different sex and menstrual stage. The percentages of the patients with smoking habit, HDL < 35 mg/dl, obesity, sedentary life, and type A personality were higher in males than those in females. The percentage of the patients with HDL ≧ 60 mg/dl was higher in premenopausal women. Hyperuricemia seemed to be more common in the female patients. There was no difference of the existence of vascular diseases among the 3 groups.

Table 1. Clinical Characteristics of the Patients

Characteristics	Male (n=58)	Premenopausal Female (n=26)	Postmenopausal Female (n=48)	p
Age (year)	51.8±11.5	46.2±5.5	59.8±7.9	<0.0001
Family history	12 (21%)	8 (31%)	8 (17%)	NS
Hypertension	14 (24%)	8 (31%)	14 (29%)	NS
Smoking	12 (21%)	0 (0%)	2 (4%)	<0.005
Diabetes mellitus	10 (17%)	0 (0%)	10 (21%)	<0.05
HDL < 35 mg/dl	18 (31%)	2 (8%)	0 (0%)	<0.0001
HDL ≧ 60 mg/dl	4 (7%)	14 (54%)	14 (29%)	0.0001
Obesity	10 (17%)	0 (0%)	4 (8%)	<0.05
Sedentary life	14 (24%)	0 (0%)	2 (2%)	<0.001
Personality type A	16 (28%)	0 (0%)	4 (8%)	0.001
Hyperuricemia	6 (10%)	6 (23%)	18 (38%)	0.005
Vascular disease	20 (34%)	8 (31%)	10 (21%)	NS

Biochemistry and Fluorophotometric Results The biochemistry and fluorophotometric data were summarized according to different sex and menstrual stage in Table 2. The premenopausal patients had the lowest serum total cholesterol, LDL-cholesterol, TG, T-CHO/HDL ratio, LDL/HDL ratio, TG/LDL ratio, glucose, uric acid levels and the highest HDL-cholesterol level. With the same trend, the F60 value was the lowest in premenopausal females, higher in males, and the highest in postmenopausal patients.

Table 2. The Biochemistry and Fluorophotometric Data of the Patients

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Parameters	Male (n=58)	Premenopausal Female (n=26)	Postmenopausal Female (n=48)	p
T-CHO (mg/dl)	231.1±45.7	229.5±26.6	266.3±62.6	<0.001
HDL (mg/dl)	40.4±10.7	56.2±13.7	53.7±10.5	<0.0001
LDL (mg/dl)	148.9±45.6	147.4±29.2	169.8±66.8	NS
TG (mg/dl)	287.7±278.5	117.5±60.1	205.4±149.6	<0.005
T-CHO/HDL	6.0±1.4	4.4±1.4	5.1±1.5	<0.0001
LDL/HDL	3.8±1.1	2.8±1.3	3.2±1.4	<0.005
TG/HDL	8.0±8.6	2.3±1.5	4.2±3.7	<0.0005
Glucose (mg/dl)	103.0±32.5	90.2±9.2	95.5±16.7	0.059
HbA1c (mg/dl)	5.9±1.5	5.2±0.8	5.7±1.1	NS
UA (mg/dl)	6.3±1.4	5.1±1.9	6.1±1.9	<0.01
F60 (ng/ml)	94.0±30.7	73.5±24.5	103.4±40.7	<0.005

Univariate Determinants of the Vascular Permeability Table 3 shows the F60 value in the patients with or without various risk or protective factors and atherosclerotic vascular disease by univariate analysis. The F60 value seemed to be higher in the aged patients, those with diabetes mellitus, or those with vascular diseases. However, the F60 value was lower in the patients with HDL ≥ 60 mg/dl.

Table 3. The F60 Value in the Patients With or Without Various Risk or Protective Factors or Atherosclerotic Vascular Diseases

Characteristics	With	Without	p
Aged	103.3±38.5	77.3±20.6	<0.0001
Family history	87.8±34.1	94.5±35.2	NS
Hypertension	93.4±34.2	92.9±35.4	NS
Smoking	95.3±25.0	92.8±36.1	NS
Diabetes mellitus	117.8±35.4	88.7±33.1	<0.001
HDL < 35 mg/dl	98.1±28.3	92.1±36.1	NS
HDL ≥ 60 mg/dl	78.4±26.9	97.6±36.0	<0.01

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Obesity	76.4±19.3	95.1±35.9	NS
Sedentary life	86.9±23.7	94.0±36.3	NS
Personality type A	97.8±30.2	92.2±35.8	NS
Hyperuricemia	97.8±44.4	91.9±32.2	NS
Vascular disease	107.0±36.4	87.3±32.8	<0.01

Table 4 shows the simple correlation between the F60 value and the patient‘s age, total risk factor number, and various serum biochemistry or lipid levels. The F60 value was positively correlated with the patients’ age, number of risk factors, cholesterol , LDL, T-CHO/HDL, LDL/HDL, glucose, and HbA1c levels, however, was negatively correlated with the HDL level.

Table 4 The Simple Correlation Between the F60 Value and the Patient‘s Age, Number of Total Risk Factors, and Various Serum Biochemistry or Lipid Levels

Parameters	Correlation coefficient	p
Age	0.32	<0.001
Number of risk factors	0.37	<0.001
T-CHO	0.50	<0.001
HDL	-0.18	<0.05
LDL	0.49	<0.001
TG	-0.06	NS
T-CHO/HDL	0.51	<0.001
LDL/HDL	0.57	<0.001
TG/HDL	-0.03	NS
Glucose	0.27	<0.01
HbA1c	0.30	<0.01
UA	0.02	NS

Multivariate Determinants of the Vascular Permeability Table 5 shows the independent determinants for the increased F60 value by multivariate stepwise linear regression analysis. It appeared that the F60 value was significantly correlated with the LDL/HDL ratio, age, diabetes mellitus, and cholesterol level. Other risk factors or the serum TG level were not significant

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independent predictors for the F60 value.

Table 5 The Independent Determinants for the Increased F60 Value by Multivariate Analysis

Variable	Beta	p
Variable in the Equation		
LDL/HDL ratio	0.461	0.0000
Age	0.173	0.0197
DM	0.301	0.0000
Cholesterol	0.238	0.0052
Constant		0.0667
Variable not in the Equation		
Triglyceride	-0.026	0.68
Sex	0.076	0.29
Family history	-0.013	0.84
Hypertension	0.065	0.34
Current smoker	0.013	0.85

DISCUSSIONS

Hypercholesterolemia is closely related to atherosclerosis and is one of the most important coronary risk factors.[17] Besides smooth muscle cell proliferation and accumulation of connective tissue matrix, changes in endothelial permeability is one of the major common pathogenic mechanisms in atherosclerosis.[1,2] Previously, the arterial transfer process in humans could be only studied by the histopathological methods when the patients died or underwent arterial surgery.[3,4,5,6,7] There is still no noninvasive method to measure the vascular permeability at the present time. In this study, we have shown that the vascular permeability could be evaluated noninvasively by fluorophotometry in human beings.

In this study, using univariate analysis, the F60 value was found to be higher in the patients with vascular diseases than those without. Many previous studies have shown that the blood-retinal barrier could be damaged in the patients with diabetes mellitus or systemic hypertension, both of

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which are major risk factors for atherosclerotic vascular diseases. In our previous study,[16] we used fluorophotometry to evaluate the vascular endothelial integrity in vivo in hyperlipidemic rabbits. The vascular permeability change correlated well with the serum cholesterol level and occurred in the very early stage of atherosclerosis. Moreover, this alteration of vascular permeability preceded the visual evidence of atheromatous plaque. That is, the vascular permeability change played an important role in the pathogenesis of vascular atherosclerosis[1,2] and could be modified by many risk factors and disease process.

We have shown in this study that in human beings, the F60 value could be determined by several factors. The F60 value was the lowest in the premenopausal female, higher in the male, and the highest in the postmenopausal patients. It was higher in the aged patients or those with diabetes mellitus. However, the F60 value was lower in the patients with HDL $\geqq$60 mg/dl. Moreover, the F60 value correlated positively with the patients' age, number of risk factors, cholesterol, LDL, T-CHO/HDL, LDL/HDL, glucose, and HbA1c levels, and correlated negatively with the HDL level. All of these are well known risk or protective factors for the development of atherosclerosis. Using multivariate analysis, in hyperlipidemic patients, the independent determinants of the F60 value were the LDL/HDL ratio, age, diabetes mellitus, and serum total cholesterol level. It implied that the vascular permeability was determined not only by one but by multiple factors, and these factors aggravated the development of atherosclerosis maybe through the effect on the vascular permeability. Because there was a high correlation between the severity of iridic and aortic atherosclerosis as well as vascular wall contents of cholesterol in animal experimental model, [8] changes of the iridic vessels in the eye would be a good indicator of the general status of the vascular system. However, this needs further large-scale and long-term follow-up study to validate the feasibility of this examination.

In conclusion, taking the advantage of the corneal clarity, the iris vasculature is one of the most easily assessed vessels all-over the body. The vascular permeability could be evaluated noninvasively by fluorophotometry. It was higher in the patients with vascular atherosclerosis and was independently determined by the patients' lipid profile, age, and the existence of diabetes mellitus. Changes of the iridic vessels in the eye might be a good window of the general status of the vascular atherosclerosis.

REFERENCES

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1. Simionescu N, Simionescu M, Palade GE: Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. J Cell Biol 1975;64:586-607.
2. Huttner I, Boutet M, More RH: Studies on protein passage through arterial endothelium. I. Structural correlated of permeability in rat arterial endothelium. Lab Invest 1973;28:672-677.
3. Simionescu N, Simionescu M, Palade GE: Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. J Cell Biol 1975;64:586-607.
4. Renkin EM: Multiple pathways of capillary permeability. Cir Res 1977;41:735-743.
5. Biggs, M. W., D. Kritchevsky, D. Colman, J. W. Gofman , H. B. Jones, F. T. Lindgren, G. Hyde, and T. P. Lyon. 1952. Observations on the fate of ingested cholesterol in man. Circulation. 6:359-366.
6. Field, H., L. Swell, R. E. Schools, and C. R. Treadwell. 1960. Dynamic aspects of cholesterol metabilism in different areas of the aorta and other tissues in man and their relationship to atherosclerosis. Circulation. 22;547-558.
7. Jagannathan,S. N., W. E. Connor, W. H. Baker, and A. K. Bhattacharyya. 1974. The turnover of cholessterol in human atherosclerotic arteries. J. Clin. Invest. 54:366-377.
8. Roscoe HG, Vogel AW: Lipid changes in the eye concomitant with the development of atherosclerosis in the aorta in the rabbit. Circulation Res 1968;23:633-643.
9. Kottow MH, Hendrickson P: Iris angiography in cystoid macular edema after cataract extraction. Arch Ophthalmol 1975;61:487-493.
10. Easty D, Dallas N, O'Malley R: Aphakic macular oedema following prosthetic lens implantation. Br J Ophthalmol 1977;61:321-326.
11. Miyake K: Prevention of cystoid macular edema after lens extraction by topical indomethacin: II. A control study in bilateral extraction. Jpn J Ophthalmol 1978;22:80-94.
12. Sawa M, Tsurimaki Y, Tsuru T, Shimizu H: New quantitative method to determine protein concentration and cell number in aqueous in vivo. Jpn J Ophthalmol 1988;32:132-42.
13. Araie M, Sawa M, Takase M: Effect of topical indomethacin on the blood-aqueous barrier after intracapsular extraction of senile cataract: A fluorophotometric study. Jpn J Ophthalmol 1981;25:237-247.
14. Sanders DR, Kraff MC, Lieberman HL, Peyman GA, Tarabishy S: Breakdown and reestablishment of the blood-aqueous barrier with implant surgery. Arch

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Ophthalmol 1982;100:588-590.
15. Maurice DM: A new objective fluorophotometer. Exp Eye Res 1963;2:33-38.
16. Wu CC, Chang SW, Chen MS, Lee YT. Early change of vascular permeability in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol. 1995;15:529-533.
17. Simons LA: Interrelations of lipids and lipoproteins with coronary artery disease mortality in 19 countries. Am J Cardiol 1986;57:5G-10G.
18. Allain LA, Poon LS, Chan LSG, Richmond W, Fu PL: Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-475.
19. McGown MW, Artiss JD, Strandbergh DR, Zak B: A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem 1983;29:538-542.
20. Miyake K, Asakura M, Maekubo K: Consensual reactions of human blood-aqueous barrier to implant operations. Arch Ophthalmol 1984;102:558-561.
21. Fearnly IR, Spalton DJ, Smith SE: Anterior segment fluorophotometry in acute anterior uveitis. Arch Ophthalmol 1987;105:1550-1555.

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