〔Original〕

Serum Lipoprotein Disc Electrophoretic Patterns and Ischemic Heart Disease*

By

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Introduction

Owing to the advances in lipid and lipoprotein chemistries in the past few decades, translation of hyperlipidemia into hyperlipoproteinemia which is the most adequate expression of hyperlipidemic state in living animals become possible. Fredrickson and his associates¹⁾ have made successfully an integrated approach to various forms of familial hyperlipoproteinemia from clinical and genetic view points and had proposed the five phenotypes of familial hyperlipoproteinemia. The phenotyping system was modified later by WHO committee²⁾ and extended to nonfamilial hyperlipoproteinemia. In this modification, type II phenotype was subdivided into two types; II-A and II-B.

Early in 1957, Besterman³⁾ found out high occurrence of abnormal lipoprotein (pre-beta lipoprotein in paper electrophoresis) among ischemic heart patients. Many other reports including our previous work⁴⁾ have confirmed his finding. Falsetti et al.⁵⁾ are probably the first who studied hyperlipoproteinemia in angiographically established coronary atherosclerotic patients. In their study, 17 of 27 (62.9%) patients showed hyperlipoproteinemia; 13 type II (not subdivided) patients and 4 type IV patients. The finding was reproducible as the number of the patients was increased to 70⁶⁾. Heinle et al.⁷⁾ reported that 68 of 126 (54%) patients with angiographically established coronary atherosclerosis showed hyperlipoproteinemia. In cases with angiographically proved normal coronary

arteries, occurrence of hyperlipoproteinemia was only 11% (7 of 66 subjects). Thirty six and 32 of 68 patients with lipoprotein abnormalities were type II and type IV, respectively. These studies strongly suggest that lipoprotein analysis would be one of the most effective means to detect atherosclerosis susceptible individuals at the earliest stage of developing coronary artery lesion.

Conventional diagnostic way of phenotyping hyperlipoproteinemia is based principally on paper electrophoretic analysis of serum lipoproteins aided by serum lipid determinations, physical findings if any, and some other laboratory findings. Lipoprotein analysis, worked usually with paper⁸⁾, agarose⁹⁾, or synthetic membrane¹⁰⁾ electrophoresis, plays a key role and is indispensable for correct identification of the phenotypes. However, limited resolution of lipoproteins which is inherent to the conventional electrophoretic methods has impeded extensive application of the phenotyping system. For example, differentiation of electrophoretic pattern of phenotype III from those of phenotype IV or II-B is not convincing frequently even with electrophorogram of the best quality, and correct differentiation of the subjects with phenotype III from others has been impossible practically.

The need for more sophisticated method of lipoprotein analysis than conventional methods has been obvious for the past years. Several methods have been explored to meet the need. Among them, polyacrylamide gel (PAG) disc type electrophoresis^{11,12)}

* 血清リポ蛋白質ディスク電気泳動像と虚血性心疾患.

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s the latest addition. PAG disc system formulated by us^{13,14} proved its practical workability in most of common clinical laboratories. Reliability of the method has been proved further with PAG block electrophoresis¹⁵ combined either with immunological or lipid chemical techniques¹⁶.

The objective of this study is to investigate hyperlipoproteinemia in ischemic heart disease with PAG disc electrophoresis added to the other conventional analytical methods. The method is effective in identifying hyperlipoproteinemia of modest to moderate degree which is encountered frequently in ischemic heart patients as well as in identifying evident hyperlipoproteinemia of familial or sporadic hyperlipoproteinemia.

Patients and Methods

Four hundred and twenty eight patients who showed any clinical signs or symptoms suggestive of ischemic heart disease were studied. Only two of them had physical and laboratory findings consistent with sporadic phenotype II and IV hyperlipoproteinemia. As a control group of lipoprotein study, 403 subjects in apparent health judged by more than two successive examinations including resting ECGs, chest X-rays and routine office laboratory tests collaborated with this study. Patients with lipid or lipoprotein disorders secondary to other diseases than atherosclerotic cardiovascular disease were not included in this study.

Clinical diagnosis of ischemic heart disease was established by reviewing present status, documented clinical histories, resting or exercise ECGs as many as available, chest X-rays of posteroanterior or multiple projections, blood chemistries and all other laboratory data of the patients. Diagnostic criteria set by WHO (ischemic heart disease)¹⁷, The Ministry of Education of Japan (coronary atherosclerosis)¹⁸, The Ministry of Health and Welfare of Japan (hypertension and ischemic heart disease)¹⁹ and Minnesota Code (resting and exercise ECG)²⁰ were consulted to establish presence or absence of ischemic heart disease.

Blood samples were obtained more than 14 hours after the last meal. Serum was used for lipid and lipoprotein analysis. Determination of serum total

cholesterol²¹ and triglyceride²² was made in all patients. Serum phospholipids²³ were determined in some of the patients. The report recently published by the study group of The Ministry of Education of Japan on age level of serum total cholesterol and triglyceride in Japanese male and female population²⁴ was consulted in evaluating serum lipid levels of the patients and control subjects.

Paper electrophoresis of serum lipoproteins was performed following the procedures by Lees et al.⁸ with modification²⁵. Whatman No. 3 MM filter paper was employed. Bovine serum albumin (Fraction V, Armour and Co., U. S. A.) and disodium EDTA were added (1% and 0.001M, respectively) to sodium barbiturate-hydrochloric acid buffer system, pH 8.6. Staining of lipoproteins on filter paper followed the method of Jencks et al.²⁶

PAG disc electrophoresis of serum lipoproteins followed the method reported previously from our laboratory¹³. Acrylamide concentration of separating gel was modified to 3.70% from the original 3.75% in this study. Reagents were obtained from the same sources as described in the previous report¹³ except lipid dye. Eastman's Sudan Black B (Eastman Kodak Co., U. S. A.) was employed in this study because of better stainability than that we had used.

Results

Reviewing lipoprotein PAG disc electrophorograms of all the subjects in this study and of approximately 2480 clinical subjects we have analysed for the past 3.5 years, the authors conceived that lipoprotein PAG disc patterns could be classified into several basic patterns. In this report, six basic lipoprotein patterns were defined empirically. They are expressed as b, bp, B, Pb and PB patterns. Electrophoretic characters of these basic lipoprotein patterns, on which identification of basic lipoprotein patterns has been made, are describable as follows (Fig. 1); b pattern: Sudan Black B stained density of α and β lipoproteins are not increased and defined as normal. Mobility of β lipoprotein is also defined as normal. Pre- β lipoprotein is not detectable. Chylomicrons are not detectable.

bp pattern: the pattern is essentially the same as b

pattern except modestly detectable pre- β lipoprotein and slightly decreased α lipoprotein.

B pattern : dense and wide β lipoprotein band is characteristic to this pattern. Mobility of β lipoprotein is slow as compared to that of b or bp patterns. Alpha lipoprotein is normal usually.

Bp pattern : moderate pre- β lipoprotein band coexists with β lipoprotein of B pattern. Alpha lipoprotein is normal or increased as compared to b pattern.

Pb pattern : markedly increased pre- β lipoprotein is characteristic to this pattern. Beta lipoprotein is always fast migrating in nature and is obviously decreased as compared with β lipoprotein of b or bp patterns. Alpha lipoprotein is markedly decreased as a rule.

PB pattern : marked increase of pre- β lipoprotein coexists with increased β lipoprotein. Beta lipoprotein band is observed as equal to, or less marked than β lipoprotein of B or Bp pattern. Mobility of β lipoprotein is faster than B pattern but slower than b pattern. Alpha lipoprotein is moderately decreased or normal.

Because of high resolution of PAG electrophoresis, further subdivision of lipoprotein patterns was possible. For example, presence of the midband lipoprotein¹⁴⁾ ("Zwischenband"¹²⁾ or intermediate lipoprotein¹³⁾, or presence of chylomicrons which are clearly detectable with the author's method would permit further subdivision. However, these subdivisions were not employed in this study, but included in any one of the six basic lipoprotein patterns.

Lipoprotein paper electrophorograms of the same patients as presented in Fig. 1 are shown in Fig. 2 (identical sera). It is obvious that PAG disc electrophorograms are consistent principally with paper electrophorograms and provide us with finer details of lipoprotein profiles than paper electrophorograms.

Serum lipid levels of six groups of 831 subjects divided by their lipoprotein pattern are compared in Table 1. As shown, total cholesterol level difference between bp and Bp patterns and between Bp and Pb patterns were statistically significant (Student "t" test), but difference between Pb and PB was not significant. Triglyceride level difference between bp and b, B

Fig. 1. Polyacrylamide gel disc electrophorograms of six basic lipoprotein patterns which are most frequently encountered among atherosclerotic cardiovascular patients.

Fig. 2. Paper electrophorograms of the patients identical to those shown in the previous figure.

and Bp, and Bp and Pb were significant. There were observed no significant differences among lipoprotein patterns regarding phospholipid level.

In the Table 2, occurrences of lipoprotein patterns in cardiovascular patients and in apparently healthy control subjects are compared. Incidences of ischemic heart disease in patient group are also shown. Occurrences of b and bp patterns in control group were 47.2% and 30.3%, respectively; approximately 77% of the control subjects had these lipoprotein patterns, while it was approximately 39% in the patient group. Occurrences of B pattern in patient group and control group was almost the same occupying 8.9% and 8.2%, respectively. Occurrences of Bp pattern in patient group and control group were 12% and 7% approximately. Occurrence of Pb pattern in the patient group

Table 1. Six basic lipoprotein patterns and serum lipids.

Lipoprotein patterns	Total cholesterol (mg/dl of serum)	Triglyceride (mg/dl of serum)	Phospholipids (mg/dl of serum)
b	168 (24)	80 (16)	175 (38)
bp	176 (25)	116 (24)	185 (41)
B	242 (42)	97 (31)	236 (40)
Bp	240 (29)	133 (32)	245 (31)
Pb	183 (36)	205 (95)	214 (43)
PB	241 (43)	218 (75)	221 (49)

Mean and SD. Statistically significant differences ($p < 0.05$) were observed between ; bp and Bp, Bp and Pb of total cholesterol ; b and bp, B and Bp, Bp and Pb of triglyceride, respectively,

Table 2. Occurrences of six basic lipoprotein patterns and incidence of ischemic heart disease.

Population	Lipoprotein patterns					
	b	bp	B	Bp	Pb	PB
Cardiovascular patients (n=428)						
Occurrence (%)	16.6	22.6	8.9	12.4	31.3	8.6
Incidence ^a (%)	25.3	29.9	64.9	60.4	42.1	40.6
Healthy control subjects (n=403)						
Occurrence (%)	47.2	30.3	8.2	7.2	7.0	0.3

a : 174 of 428 patients (40.7 %) had ischemic heart disease

were obviously higher than control group ; it was approximately 31 % in the patient group and 7 % in the control group. Occurrence of PB pattern in the patient group was approximately 9 % and it was lower than 1 % in the control group.

Incidences of ischemic heart disease in B and Bp patterns were markedly higher than others. Approximately 65 % of the patients with B patterns and 60 % of the patients with Bp pattern were ischemic heart patients. Incidences of ischemic heart disease in Pb and PB patterns were approximately 42 % and 41 %, respectively. Incidences of ischemic heart disease in the patients with b and bp patterns were approximately 25 % and 30 %, respectively.

An investigation of the relationship between lipoprotein patterns and incidence of ischemic heart disease in decadal age group gave a result suggesting that age factor is implicated in the development of ischemic heart disease associating with lipoprotein pattern. As shown in Table 3, incidence of ischemic

heart disease exceeded 50 % at the 7 th decade (61 to 70 years old) in the patients with b pattern, at the 6 th decade in the patients with bp and PB pattern, at the 5 th decade in the patients with B or Pb pattern and at the 4 th decade in the patients with Bp pattern, respectively.

Discussion

As studied with lipoprotein PAG disc electrophoretic method, six lipoprotein patterns were differentiable as basic variations of serum lipoprotein profile. Of the six basic lipoprotein patterns, B, Bp and Pb patterns are describable as hyperbeta lipoproteinemia, hyperbeta lipoproteinemia associated with moderate hyperpre-beta lipoproteinemia and hyperpre-beta lipoproteinemia, respectively. Following WHO's definition, they are phenotypes II -A, II -B and IV, respectively, though only two of approximately 830 subjects studied in this work showed physical signs and laboratory findings consistent with sporadic hyperlipoproteinemia

Table 3. Incidences of ischemic heart disease in decadal age groups of six basic lipoprotein patterns^a.

Lipoprotein pattern	Nb	Age group				
		~40	41~50	51~60	61~70	70~
b	71	0*	0.19	0.40	0.67	0.70
bp	97	0.17	0.33	0.53	0.57	0.50
B	37	0.22	0.67	0.67	0.83	1.00
Bp	53	0.53	0.46	0.62	0.71	0.50
Pb	133	0.14	0.58	0.56	0.70	1.00
PB	37	0.07	0.46	0.80	0.50	0.60

a : Incidence in an age group is expressed by dividing number of the patients with ischemic heart disease by total number of the patients in an age group of a lipoprotein pattern. b : number of the patients.

* : none had ischemic heart disease.

of the classical definition.

The prevalent lipoprotein pattern among the healthy control subjects were b and bp pattern (approximately 77 % of the subjects). Incidence of ischemic heart disease in the patients with bp pattern was not obviously higher but the earliest possible onset of ischemic heart disease was lower as compared with the patients with b pattern.

Occurrence of B and Bp patterns were low both in the control subjects and patient group. However, incidences of ischemic heart disease in those who were associated with these lipoprotein patterns were extremely high (more than 60 %) as compared with others. Incidence of ischemic heart disease in the patients with Pb pattern was lower than in the patients with B or Bp pattern, but occurrence of this pattern in the patient group was high suggesting that total number of the patients with this lipoprotein abnormality and ischemic heart disease are to be estimated as large. These findings are consistent with the studies^{3,4,6,7)} performed by means of paper electrophoretic methods.

PB pattern is a combined hyperbeta and hyperpre-beta lipoproteinemia, which is differentiable convincingly with PAG disc electrophoresis from Bp or Pb patterns. From clinical view point, incidence of ischemic heart disease in PB pattern was lower than in Bp pattern. Estimated age of the earliest possible onset of ischemic heart disease in the patients with PB pattern was higher than in those who with Bp pattern. Therefore, it seems more reasonable to con-

sider that PB pattern is an independent lipoprotein pattern from other patterns, especially from Bp or Pb patterns. The most adequate category to which PB pattern belongs seems to be "the sixth phenotype"²⁷⁾ or "the combined hyperlipoproteinemia"²⁸⁾. In this case, phenotype VI is not the sixth but should be the seventh phenotype, because phenotype II-B of WHO definition²⁾ is identifiable as Bp pattern with PAG disc electrophoresis.

Summary

Serum lipoprotein polyacrylamide gel disc electrophoresis aided by paper electrophoresis was proved to be effective to detect and identify convincingly modest to moderate hyperlipoproteinemias which are frequently encountered in atherosclerotic cardiovascular clinic. Hyperbeta lipoproteinemia with moderate increase of pre- β lipoprotein (Bp pattern, type II-B of WHO definition) showed closest association with ischemic heart disease. Premature development of ischemic heart disease in the patients with this lipoprotein pattern was evident. Hyperbeta lipoproteinemia (B pattern, type II-A of WHO definition) showed also close association with high incidence and premature development of ischemic heart disease. Hyperpre-beta lipoproteinemia (Pb pattern, type IV of WHO definition) occurred most frequently among atherosclerotic cardiovascular patients, though incidence of ischemic heart disease was lower than those with Bp or B patterns. The mixed hyperlipoproteinemia (PB pattern, the sixth phenotype) which is identifiable

most adequately with PAG disc method showed the incidence of ischemic heart disease comparable to those in Pb pattern and the occurrence comparable to those in B pattern. The sixth phenotype should be the seventh phenotype because phenotype II-B (Bp pattern) is clearly differentiable from the mixed hyperlipoproteinemia (PB pattern).

要 約

動脈硬化性病変を背景として生じる虚血性心疾患は、血清脂質ならびに血清リポ蛋白質の異常と密接に関連する。本研究の目的は古典的な血清リポ蛋白質浮紙電気泳動法に高い電気泳動的分離能を有するポリアクリルアミドゲル・ディスク電気泳動法を加えた分析を行ない、虚血性心疾患における血清リポ蛋白質の異常を一層鮮明に捉えようとするものである。

対象は心循環器系疾患に関係した愁訴を以て受診もしくは入院をした患者を中心とし、ほぼ同数の健常者が含まれている。全例につき理学所見、一般臨床生化学的検査、胸部X線検査、心電図検査、脂質化学的検査などを実施した。血清リポ蛋白質浮紙ならびにディスク電気泳動法は著者らの既報した方法に従って実施した。

対象例の血清リポ蛋白質ディスク電気泳動像は6種の基本的電気泳動像 (b, bp, B, Bp, Pb, PB と仮称された) に分類することができた。これらはそれぞれにある程度特徴的な脂質化学的性質を示していた。臨床像との関連では高Bリポ蛋白質血症状態を反映するBあるいはBpの像が集団における出現頻度は比較的低いが、虚血性心疾患の検出率は著しく高く(60~65%)、注目された。高ブレ-βリポ蛋白質血症の状態を示すPbの像は、虚血性心疾患の検出率は比較的低い(42%)が、患者集団における出現頻度が対照集団に比べて著しく高い点が注目された。PB型は最近提唱されるに至っている第VIのフェノタイプもしくは混合型高リポ蛋白質血症に相当するものと考えられるがディスク法によって適確に診断され得た。出現頻度はBあるいはBpに近く、虚血性心疾患の検出頻度はPbのそれに近い率を示した。本研究の結果からポリアクリルアミドゲル電気泳動法による血清リポ蛋白質の分析は、虚血性心疾患患者における異常の検出に有効であると結論された。

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