

Plasma fibronectin levels in ischemic heart disease

Kyung Soon Song^{a,*}, Hyun Kyung Kim^a, Wonhm Shim^b, Sun Ha Jee^c

^a Department of Clinical Pathology, College of Medicine, Yonsei University Medical Center, CPO Box 8044, Seoul, South Korea

^b Department of Internal Medicine, College of Medicine, Yonsei University Medical Center, CPO Box 8044, Seoul, South Korea

^c Graduate School of Health Science and Management, Yonsei University Medical Center, CPO Box 8044, Seoul, South Korea

Received 5 July 1999; received in revised form 14 March 2000; accepted 29 March 2000

Abstract

Fibronectin is a paradigm adhesive protein which has been implicated in the regulation of several cellular processes and cell-cell interactions. Large amounts of fibronectin have been detected in atherosclerotic plaques, while hypertension in animal models has been shown to rapidly increase fibronectin expression in arterial walls. The aim of the present study was to determine the levels of plasma fibronectin (FN) in 133 patients with ischemic heart disease and in 36 normal controls, and to investigate the possible association with blood pressure. Plasma FN levels in patients with ischemic heart disease were found to be significantly elevated (mean \pm S.D.; 46.5 ± 14.2 mg/dl) compared with the control group (38.0 ± 14.2 mg/dl) ($P = 0.002$). Plasma FN concentrations were significantly different between the hypertensive group (52.9 ± 14.5 mg/dl) and the normal blood pressure group (41.4 ± 11.8 mg/dl) among the patients with ischemic heart disease ($P < 0.001$). Plasma FN concentration was positively correlated with total cholesterol, triglyceride, systolic blood pressure and body mass index. In conclusion, the plasma fibronectin level may have pathogenetic implications in association with lipid components and blood pressure in patients with ischemic heart disease. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Fibronectin; Ischemic heart disease; Atherosclerosis; Hypertension

1. Introduction

Fibronectin (FN) is a high molecular weight glycoprotein which occurs in a soluble form in plasma and other body fluids, as well as in an insoluble form in the extracellular matrix (ECM) of most tissues [1]. It serves as a bridge between cells and the interstitial collagen meshwork, and influences diverse processes including cell growth, adhesion, migration and wound repair [2,3]. Vascular endothelial cells seem to be the major source of plasma FN, although FN is synthesized by a variety of cells in vitro, including fibroblasts, smooth muscle cells and epithelial cells [4].

Stenman et al. observed prominent immunofluorescent staining for FN in early cellular atherosclerotic lesions of human muscular arteries, which may suggest that excessive production contributes to the pathogenesis of atherosclerotic cardiovascular disease [5]. Fur-

thermore, hypertension in animal models has been shown to rapidly increase FN expression, correlating with the degree of blood pressure elevation [6–8]. The aim of this study was to determine the levels of plasma FN in ischemic heart disease and to investigate the possible association of FN with blood pressure in atherosclerosis.

2. Methods

2.1. Study groups

A total of 133 patients (male: 96, female: 37, mean age: 59.9 years, range 36–89) who underwent diagnostic cardiac catheterization were studied. According to their angiographic findings, the group consisted of 58 patients with single-vessel disease, 38 patients with double-vessel disease, and 37 patients with triple-vessel disease. Clinical diagnosis included stable angina ($n = 52$), unstable angina ($n = 61$), and acute myocardial infarct ($n = 20$). Unstable angina was defined as angina

* Corresponding author. Tel.: +82-2-361-6470; fax: +82-2-313-0956.

E-mail address: kssong@yumc.yonsei.ac.kr (K.S. Song).

pectoris of recent onset, angina with deterioration during the 4 weeks prior to admission with respect to pain intensity and duration, or anginal pain at rest or during very low physical exertion [9]. Stable angina was defined as typical chest pain on exertion, associated with horizontal or downsloping ST-segment depression of > 1.0 mm at 80 ms following the J point on an exercise test. Acute myocardial infarct based on World Health Organization criteria was defined as typical chest pain, typical enzyme patterns, and diagnostic ECG changes. A total of 59 patients were hypertensive while 74 patients did not have hypertension as judged by the criteria of the Joint National Committee and WHO/International Society of Hypertension [10]. No patient had thromboembolism, collagen disease, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, septicemia, or any other inflammatory diseases. There were no differences in the number of patients receiving drugs (aspirin, nitrates, β -blockers, calcium antagonists, ACE inhibitor or anti-platelet agents) between the hypertensive group and the normal blood pressure group (Table 1).

A total of 36 control subjects (male: 23, female: 13, mean age: 57.6 years, range 42–79), matched the patients for age (control vs patients, $P = 0.169$; normal blood pressure vs hypertension, $P = 0.376$) and gender (control vs patients, $P = 0.337$; normal blood pressure vs hypertension, $P = 0.114$), were selected from healthy volunteers. 'Healthy' was defined as being free from symptoms of heart disease and without hospitalization for any illness during the previous 5 years.

2.2. Data collection

Blood samples were drawn between 8 and 11 am while the subject was in a supine position, following 5 min of supine rest and an overnight fast. Weight was measured in kilograms and height in meters. Body mass index (BMI) was calculated as $\text{weight}/\text{height}^2$. Systolic and diastolic blood pressures were measured in a supine position after a 5-min rest. Hypertension was defined as blood pressure $> 140/90$ mmHg. Diabetes mellitus was defined as either insulin-dependent or non-insulin-dependent diabetes mellitus. Smoking habits were assessed by questionnaire and categorized as non-smoker and smoker (current and former).

2.3. Laboratory methods

Plasma samples for FN assay were obtained from 3.8% citrated venous blood and centrifuged at $3000 \times g$ for 15 min. Aliquots of separated plasma were stored at -70°C until analyzed. FN was determined by nephelometric immunoassay (Behring, Germany). Serum samples for lipid parameters were obtained after 12 h fasting and Lp(a) levels were determined by ELISA method (Boehringer Mannheim Biochemica, Germany). Total and high-density lipoprotein cholesterol (HDLc) and triglycerides were measured using the standard enzymatic method with BM/Hitachi 747 (Boehringer-Mannheim Diagnostics, Germany). Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula if triglycerides were < 400 mg/dl [11].

Table 1
Characteristics of study groups^a

Characteristics	Controls ($n = 36$)	Ischemic heart disease		
		Total ($n = 133$)	Normal blood pressure ($n = 74$)	Hypertension ($n = 59$)
<i>Age (yr)</i>				
Mean \pm S.D.	57.6 \pm 7.6	59.9 \pm 9.3	59.3 \pm 9.9	60.7 \pm 8.6
Range	42–79	36–89	36–89	37–79
<i>Men/women (n)</i>				
	23/13	96/37	55/19	41/18
<i>Smokers (n)</i>				
	23	79	48	31
<i>Body mass index (kg/m²)</i>				
	23.7 \pm 2.3	24.5 \pm 3.2	24.3 \pm 2.5	24.7 \pm 4.0
<i>Diabetes mellitus (n)</i>				
	5*	46	22	24
<i>Hypertension (n)</i>				
	13	59	0	59
<i>Clinical diagnosis</i>				
Stable angina	0	52	28	24
Unstable angina	0	61	33	28
Acute myocardial infarct	0	20	13	7
<i>Medication used</i>				
β blocker	0	58	31	27
Anti-platelet agent	0	90	51	39
Calcium antagonist	0	85	48	37
Aspirin	0	123	71	52
ACE inhibitor	0	25	14	11

^a * $P < 0.05$ versus total ischemic heart disease group. Values are expressed as mean \pm S.D. or number of individuals.

Table 2

Plasma FN levels (mean \pm S.D.) in patients with ischemic heart disease and controls^a

Groups	<i>n</i>	FN (mg/dl)	<i>P</i> value
Ischemic heart disease	133	46.5 \pm 14.2	0.002*
Normal blood pressure	74	41.4 \pm 11.8	<0.001
Hypertension	59	52.9 \pm 14.5	
Controls	36	38.0 \pm 14.2	
Normal blood pressure	23	34.3 \pm 11.0	0.038
Hypertension	13	44.5 \pm 17.3	

^a **P* value versus controls.

2.4. Statistical methods

Tests of normality were performed to verify the distribution of study variables. Student's *t*-test was used to evaluate differences in continuous variables between patient and control groups or normal blood pressure and hypertension groups. The chi-square test was performed for discrete variables. ANOVA analysis was used for comparison among subgroups of patients. Pearson correlation coefficient was used for the correlation analysis. Odds ratios, as measures of relative risk, were estimated using logistic regression analyses, and 95% confidence intervals (CI) were computed. The plasma FN level was categorized as (1) < 40 mg/dl; (2) 40–55 mg/dl; and (3) > 55 mg/dl according to its distribution. All statistical tests were two-sided and were considered to be statistically significant at a *P* value < 0.05.

3. Results

Plasma FN levels in patients with ischemic heart disease were found to be significantly elevated (mean \pm S.D.; 46.5 \pm 14.2 mg/dl), compared to the control group (38.0 \pm 14.2 mg/dl) (*P* = 0.002, Table 2, Fig. 1). Plasma FN concentrations were significantly different between

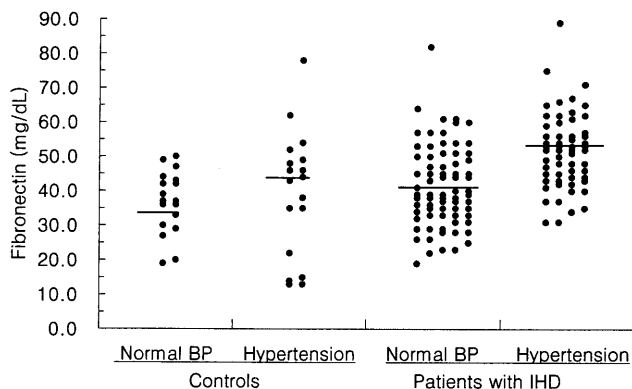


Fig. 1. Distribution of fibronectin among groups. Each point represents a value from a single patient or control. Bars indicate the mean concentration for each group. BP, blood pressure; IHD, ischemic heart disease.

Table 3

Correlation coefficients for association with plasma FN and lipids^a

Variable	Correlation coefficient	<i>P</i> value
Lp(a)	0.028	0.739
Total cholesterol	0.243	0.002*
Triglyceride	0.474	<0.001*
HDL cholesterol	-0.148	0.062
LDL cholesterol	0.089	0.265
Age	0.029	0.707
Body mass index	0.174	0.049*
Systolic blood pressure ^b	0.269	0.001*
Diastolic blood pressure ^b	0.146	0.066

^a HDL, high density lipoprotein; LDL, low density lipoprotein **P* < 0.05.

^b Blood pressure measured at the time of blood collection for laboratory tests including FN.

the hypertensive group (52.9 \pm 14.5 mg/dl) and the normal blood pressure group (41.4 \pm 11.8 mg/dl) among the patients with ischemic heart disease (*P* < 0.001, Table 2, Fig. 1). And among healthy controls, plasma FN levels in the hypertensive group (44.5 \pm 17.3 mg/dl) were significantly higher (*P* = 0.038) than those in the normal blood pressure group (34.3 \pm 11.0 mg/dl). The plasma FN levels in the unstable angina group, the stable angina group and the acute myocardial infarction group were 51.1 \pm 15.2, 42.6 \pm 12.2, and 42.6 \pm 12.2 mg/dl, respectively. The plasma FN levels in the unstable angina group were significantly higher (*P* < 0.01) than those in the stable angina and acute myocardial infarction groups. The plasma FN levels were not associated with angiographic findings (one vessel, 46.4 \pm 15.3 mg/dl; two vessels, 43.9 \pm 13.2 mg/dl; three vessels, 48.7 \pm 13.0 mg/dl, *P* = 0.348). Plasma FN levels were not correlated with serum Lp(a) as well as HDL or LDL cholesterol, but were positively correlated with total cholesterol, triglyceride, systolic blood pressure and BMI in the patient group (Table 3). The odds ratios for patients in each of the categorized FN levels are shown in Table 4. The unadjusted odds ratio for ischemic heart disease in the highest quadrant (> 55 mg/dl) versus the lowest quadrant of FN was 2.55 (95% CI, 1.19–5.47) and the multivariate-adjusted odds ratio was 4.57 (95% CI, 1.55–13.47).

Table 4

Odds ratios for ischemic heart disease according to plasma FN levels

FN	Univariate	Multivariate ^a	95% CI
< 40 mg/dl	1 ^b	1 ^b	
40–55 mg/dl	1.70	2.77	1.11–6.94
> 55 mg/dl	2.55	4.57	1.55–13.47

^a Adjusted for age, sex, diabetes, smoking, hypertension and body mass index.

^b Reference category.

4. Discussion

In this study, plasma FN levels were investigated in ischemic heart disease. An increased level of plasma FN, compared to the control group, was found in patients with ischemic heart disease. Furthermore, unstable angina patients had higher concentrations of plasma FN than patients with stable angina and acute myocardial infarct. Similarly, some studies showed increased plasma FN in ischemic heart disease [12,13]. Gavrilov et al. found that in patients with an unstable angina, the plasma FN level was found to be high [13]. The high concentrations of plasma FN in unstable angina patients may be related to the disruption of coronary atherosclerotic plaques with exposure of the atheroma to the blood flow. We determined that plasma FN levels > 55 mg/dl show a nearly fivefold increased risk of ischemic heart disease compared to the reference category (FN < 40 mg/dl) after adjustment for age, sex, diabetes, smoking, BMI and hypertension.

Hypertension in animal models has been shown to rapidly increase in FN expression, correlating with the degree of blood pressure elevation; this expression is reversed by normalization of the blood pressure [6,8]. Several investigators have reported that the plasma FN level was elevated in a group of preeclamptic patients and that increased total plasma FN levels predicted the development of gestational hypertension with a sensitivity of 96% and a specificity of 94% [14,15]. Until recently, it was assumed that higher plasma FN levels in pregnancy-induced hypertensive disorder were caused mainly by endothelial cell activation and/or dysfunction and subsequent repair processes [16].

The values of hypertensive patients with ischemic heart disease were higher than those of normotensive subjects in this study. Hypertensive individuals in the control group also showed increased plasma FN levels, compared with normal blood pressure individuals. We observed a correlation between elevated plasma FN levels and systolic blood pressure. These findings suggest that plasma FN could in part be implicated in the pathogenesis of hypertension. However, we could not determine how much total plasma FN is derived from systemic endothelial cells [4] and how much is derived from liver sinusoids [17], trophoblast [18] or increased platelet activation [19].

We observed that plasma FN was positively correlated with serum total cholesterol and triglyceride concentrations. Previously, plasma FN was reported to be positively correlated with body mass index and serum triglyceride concentration in overweight patients, suggesting that metabolic disturbances related to body obesity may lead to enhanced hepatic secretion of very low density lipoprotein (VLDL) and several plasma proteins including FN [20]. In humans, 60–70% of cholesterol is transported by low density lipoprotein

(LDL) and LDL has been shown to stimulate the production of the matrix glycoprotein FN and chemoattractant expression [21]. In close accord with this finding, we have demonstrated that there was a positive correlation between plasma FN and total cholesterol.

Previous reports have shown that plasma FN values are significantly lower in women than in men [22]. In our study, no significant differences in plasma FN were observed between male and female among each age group as well as between smokers and non-smokers (data not shown). Similarly, aspirin may also be considered for its effect as an anti-inflammatory substance. However, the fibronectin levels in aspirin-consuming patients (mean: 46.6 mg/dl) were similar to those in non-consuming patients (mean: 45.2 mg/dl). Therefore, aspirin does not seem to affect the plasma fibronectin levels.

Although the plasma FN levels were significantly different between hypertension and normotension, and between the control and ischemic heart disease groups, as shown in Fig. 1, a rather wide distribution of individual FN levels in each group was observed. Plasma FN levels may be influenced not only by hypertension and/or coronary artery disease, but also by BMI, total cholesterol, triglyceride (as shown in the Table 3) and other unknown factors. Compared with the patient group, the number of controls is relatively small, which is the limitation in our study. Further study based on a large population and longitudinal study are needed to confirm our results.

Although the exact mechanism of elevated FN in ischemic heart disease and hypertension is not clear, it is considered that the plasma FN level may have pathogenic implications in association with lipid components in patients with ischemic heart disease and with hypertension. However, further studies concerning the impact of lipid mediators and FN deposition on the pathogenesis of cardiovascular disease are necessary.

Acknowledgements

This study was supported in part from a HMP grant (No. HMP-98-1-004) of the 98 Good Health Research and Development Project, the Ministry of Health and Welfare, Republic of Korea.

References

- [1] Colman RW, Hirsh J, Marder VJ, Salzman EW. Hemostasis and Thrombosis. Philadelphia: JB Lippincott Company, 1994:751–2.
- [2] Pearstein E. Plasma membrane glycoprotein which mediates adhesion of fibroblasts to collagen. *Nature* 1976;262:497–500.
- [3] Humphries MJ, Obara M, Olden K, Yamada KM. Role of fibronectin in adhesion, migration and metastasis. *Cancer Invest* 1989;7:373–93.

- [4] Mosesson MW, Amrani DL. The structure and biological activities of plasma fibronectin. *Blood* 1980;56:145–58.
- [5] Stenman S, Von Smitten K, Vaheri A. fibronectin and atherosclerosis. *Acta Med Scand* 1980;642:165–70.
- [6] Takasaki I, Chobanian AV, Sarzani R, Brecher P. Effect of hypertension on fibronectin expression on the rat aorta. *J Biol Chem* 1990;265:21935–9.
- [7] Saouaf R, Takasaki I, Eastman E, Chobanian AV, Brecher P. fibronectin biosynthesis in the rat aorta in vitro: change due to experimental hypertension. *J Clin Invest* 1991;88:1182–9.
- [8] Takasaki I, Chobanian AV, Mamuya WS, Brecher P. Hypertension induces alternatively spliced forms of fibronectin in rat aorta. *Hypertension* 1992;20:20–5.
- [9] Braunwald E. Unstable angina: a classification. *Circulation* 1989;80:410–4.
- [10] Hollenberg NK. Summary of the Joint National Committee (JNC)-V and WHO/International Society of Hypertension (ISH) special reports. In: Holleberg NK, editor. *Hypertension: Mechanism and Therapy*. Philadelphia: Current Medicine, 1995:13.1–13.16.
- [11] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [12] Sonmez H, Suer S, Kokoglu E, Dirican A, Ulutin T, Ucisik N, Ulutin ON. The importance of Lp(a)-fibronectin interaction in atherogenesis. *Haematologia* 1997;28:149–53.
- [13] Gavrillov OK, Lekokhmakher SS, Magomedov NG, Dobashina AN, Besprozvannyi AB, Shakhova NI. Content of fibronectin in plasma of patients with ischemic heart disease and possibilities of its correction by extracorporeal methods. *Kardiologiya* 1995;35:18–20.
- [14] Stubbs TM, Lazarchick J, Horger EO. Plasma fibronectin levels in preeclampsia: a possible biochemical marker for vascular endothelial damage. *Am J Obstet Gynecol* 1984;150:885–7.
- [15] Ballegeer VC, Spitz B, Kieckens L, Moreau H, Van Assche A, Collen D. Predictive value of increased plasma levels of fibronectin in gestational hypertension. *Am J Obstet Gynecol* 1989;161:432–6.
- [16] Paarlberg KM, Dejong CD, Van Geijn HP, Van Kamp GJ, Heinen AG, Dekker GA. Total plasma fibronectin as a marker of pregnancy-induced hypertensive disorders: a longitudinal study. *Obstet Gynecol* 1998;91:383–8.
- [17] Rieder H, Ramadori G, Dienes HP, Meyer zum Buschenfelde KH. Sinusoidal endothelial cells form guinea pig liver synthesizes and secretes cellular fibronectin in vitro. *Hepatology* 1987;7:856–64.
- [18] Chou-Rong Zhu B, Fisher SF, Pande H, Calaycay J, Shively JE, Laine RA. Human placental(fetal) fibronectin: increased glycosylation and higher protease resistance than plasma fibronectin. *J Biol Chem* 1984;259:3962–70.
- [19] Konijnenberg A, Stokkers EW, Van der Post JAM, Schaap MCL, Boer K, Bleker OP. Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell adhesion molecules. *Am J Obstet Gynecol* 1997;176:461–9.
- [20] Cucuianu M, Bodizs G, Duncea I, Colhon D. Plasma fibronectin in overweight men and women: correlation with serum triglyceride levels and serum cholinesterase activity. *Blood Coagul Fibrinolysis* 1996;7:779–85.
- [21] Rovin BH, Tan LC. LDL stimulates mesengial fibronectin production and chemoattractant expression. *Kidney Int* 1993;43:218–25.
- [22] Lefevre A, Mazurier C, Goudemand M. Comparative study of 3 techniques for the determination of plasma fibronectin. Results in the normal subject. *Rev Fr Transfus Immunohematol* 1983;26:135–45.