*Hypertension*

# The Serum Concentrations of Procollagen Propeptides in Hypertensive Patients with or Without Diabetes

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***Background:*** The serum concentrations of procollagen propeptides are valuable markers of myocardium collagen synthesis and turnover in hypertensive patients. The aim of our study was to evaluate the effect of diabetes mellitus on serum concentrations of procollagen propeptides in hypertensive patients.

***Methods:*** Sixty-three subjects admitted for coronary angiography were enrolled. All of the patients received analysis of the serum levels of the amino-terminal propeptide of types I and III procollagen (PINP and PIIINP).

***Results:*** Patients were divided into 3 groups according to the presence or absence of hypertension and diabetes; these were: 9 patients with both hypertension and diabetes (group 1), 38 patients with hypertension but without diabetes (group 2), and 16 normotensive patients without diabetes (group 3). Group 2 patients had higher serum PINP levels than group 1 and group 3 patients (p = 0.023 and p = 0.004, respectively). No significant difference of serum PINP level was noted between group 1 and group 3. However, the serum PIIINP level in group 1 patients was significantly higher than those in group 2 and group 3 (p = 0.005 and p < 0.001, respectively), and the serum PIIINP level between group 2 and group 3 showed no significant difference.

***Conclusion:*** Diabetic status influenced serum PINP and PIIINP levels differently in hypertensive patients.

**Key Words:**   PINP • PIIINP • Myocardial fibrosis • Hypertension • Diabetes mellitus

## INTRODUCTION

The extra-cellular matrix (ECM) of heart consists mainly of types I and III collagen.[1] The serum markers of collagen I and III are amino-terminal propeptide of type I and III procollagen (PINP, PIIINP) and carboxyl terminal propeptide of type I procollagen (PICP). They are all valuable markers of myocardium collagen synthesis and turnover.[2-4] These markers are useful tools for cardiac tissue repair and fibrosis monitoring,[5] both in experimental[6] and clinical practice[4,7,8] models. Excess myocardial collagen deposition has been related to several pathological conditions, including coronary artery disease,[9] cardiac hypertrophy,[3,10] myocardial hibernation,[11] myocardial infarction (MI)[12] and congestive heart failure.[13] In hypertensive patients, serum markers of type I collagen synthesis (PINP and PICP) are especially useful. They not only reflect the severity of myocardial fibrosis, but also the diastolic dysfunction in hypertensive patients.[14]

Hypertension is a component of the insulin resistance-related metabolic syndrome.[15] Diabetes mellitus increases myocardial fibrosis in animal model[16,17] and

human study.[18] However, in a recent study, François et al. showed a higher PIIINP but a lower PINP level in patients with both hypertension and diabetes, comparing with the controlled group.[19] Diabetes mellitus itself seemed to have different effect on the serum PINP and PIIINP levels. The aim of our study was to evaluate the effect of diabetes on serum markers of collagen I and III in hypertensive patients.

## PATIENTS AND METHODS

### Study population

We prospectively studied 63 consecutive patients aged from 50 to 75 year old, 37 males and 26 females. These patients were admitted for coronary angiography due to positive stress test. Patients were divided into 3 groups according to the presence of hypertension and diabetes. These were: 9 patients with both hypertension and diabetes (group 1), 38 patients with hypertension but without diabetes (group 2), and 16 normotensive patients without diabetes (group 3). Conditions associated with elevated serum concentrations of PINP and PIIINP, including chronic liver disease, pulmonary fibrosis, rheumatoid arthritis, and extensive wounds were excluded after complete medical examination. Additional exclusion criteria were significant valvular heart disease, coronary intervention within the previous 3 months, leukocytosis, and active infection. Blood pressure measurements were performed by trained technicians or nurses with a mercury sphygmomanometer, and the first and fifth Korotkoff sounds were recorded to represent the systolic and diastolic pressures. Three measurements were obtained on each occasion, at 5-minute intervals, and averaged. Hypertension was said to be present if the systolic blood pressure was 140 or diastolic blood pressure 90 mm Hg or there was use of medication for hypertension (not including the usage of angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker for diabetic nephropathy patients or β-blocker for normotensive patients with mitral valve prolapse or coronary artery disease). Diabetes mellitus was defined as a blood glucose level of 126 mg/dL or higher after an overnight fast or use of medication for diabetes mellitus. Hyperlipidemia was defined as a total cholesterol level ≧ 240 mg/dL or total triglyceride ≧ 200 mg/dL or use of

medication for hyperlipidemia. Coronary artery disease (CAD) was defined as ≧ 50% coronary artery diameter stenosis detected by diagnostic coronary angiography. The study was approved by the ethics committee of the National Taiwan University Hospital, and all subjects gave informed consent.

### Blood sampling and determination of PINP and PIIINP

Blood sampling was obtained from peripheral vein or percutaneous artery sheath before catheterization. Serum samples to determine PINP and PIIINP were stored at -40 °C until assay. Serum PINP was determined by a rapid equilibrium radioimmunoassay using commercial antisera specifically directed against the amino-terminal propeptide (Orion Diagnostica, Espoo, Finland). The intra-and interassay coefficients of variation were < 7%. The sensitivity of this method was 2 μg/L. The reference range of serum PINP was 20-76 μg/L in men and 19-84 μg/L for women. Serum PIIINP was determtained by a coated-tube radioimmunoassay as described using commercial antisera specifically directed against the amino-terminal propeptide (Orion Diagnostica, Espoo, Finland). The intra- and interassay coefficients of variation of serum PIIINP were < 5%. The sensitivity for PIIINP was 0.3 μg/L. The reference range of serum PIIINP was 2.3-6.4 μg/L.

### Statistical analysis

Continuous data was expressed by mean ± standard deviation. Differences between proportions were assessed by chi-square test or Fisher exact test. Differences between two groups were tested by Student's *t* tests. We used one-way ANOVA method with subgroup post-hoc analysis (LSD) to compare continuous variables among three groups. A p-value < 0.05 was considered significant. All analysis was performed by using SPSS 10.0 manager software.

## RESULTS

The clinical data and echocardiographic results are summarized in Table 1. No significant difference was found in age, sex, hyperlipidemia, serum creatinine level, left ventricular ejection fraction, left ventricular

**Table 1.** Patient characteristics in all groups of study patients

|  | Group 1 (n = 9) | Group 2 (n = 38) | Group 3 (n = 16) | P value |
|---|---|---|---|---|
| Age (years) | 67.0 ± 4.7 | 64.2 ± 8.5 | 60.9 ± 6.0 | 0.140 |
| Gender* | 6 (67%) | 19 (50%) | 12 (75%) | 0.212 |
| LVEF (%) | 63.9 ± 6.7 | 68.3 ± 7.3 | 69.1 ± 5.2 | 0.160 |
| LVMI (g/m2) | 124.4 ± 29.7 | 122.7 ± 31.4 | 125.3 ± 31.6 | 0.962 |
| CAD | 6 (67%)[a] | 11 (29%) | 6 (38%) | 0.110 |
| Hyperlipidemia | 6 (67%) | 24 (45%) | 6 (38%) | 0.240 |
| Creatinine | 1.0 ± 0.3 | 1.1 ± 0.2 | 1.1 ± 0.2 | 0.944 |
| Medication |  |  |  |  |
| ACEI/ARB | 4 (44%) | 19 (50%)[b] | 2 (12.5%) | 0.034 |
| B-blocker | 6 (67%) | 15 (39%) | 5 (31%) | 0.219 |
| Statin | 3 (33%) | 10 (26%) | 3 (19%) | 0.719 |

*Expressed as the male gender number.
LVEF = left ventricular ejection fraction; LVMI = left ventricular mass index; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CAD = coronary artery disease.
[a] $p < 0.05$ between group 1 and group 2; [b] $p < 0.05$ between group 2 and group 3.

mass index, β-blocker, and statin usage among the three groups. The group 1 patients had higher CAD percentage than group 2 patients, but there was no significant difference of the CAD ratio between group 1 and group 3. Angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker was prescribed more in group 2 than in group 3 patients (50% v.s 12.5%, $p < 0.05$).

The result of serum PINP and PIIINP levels among the 3 groups is shown in Table 2. The PINP levels were 33.6 ± 13.3 μg/L, 50.2 ± 21.6 μg/L, and 33.0 ± 15.6 μg/L, respectively, in group 1, group 2, and group 3. The serum PINP level was significantly higher in group 2 than those in group 1 ($p = 0.023$) and group 3 ($p = 0.004$). No significant difference of the PINP level was noted between group 1 and group 3. The PIIINP levels were 5.9 ± 2.7 μg/L, 4.3 ± 1.3 μg/L, and 3.5 ± 0.7 μg/L, respectively, in group 1, group 2, and group 3 patients. The group 1 (combined hypertension and diabetes) pa-

tients had significantly higher serum PIIINP level than that in group 2 ($p = 0.005$) and group 3 ($p < 0.001$) patients. The PIIINP level in group 2 patients was non-significantly higher than that in group 3 patients ($p = 0.1$). Among the hypertensive patients, no significant difference of the PINP and PIIINP levels was noted whether using ACEI/ARB or not (Table 3).

## DISCUSSION

The collagen synthesis in ECM of heart increases in certain conditions, including cardiac hypertrophy,[3,10] myocardial hibernation,[11] myocardial infarction (MI),[12] and congestive heart failure.[13] Type I and type III collagen accumulation raises myocardial stiffness, which leads to cardiac structure remodelling and is the major determinant of cardiac diastolic dysfunction.[20] Serum markers of both type I and type III collagen turnover were shown to be a useful marker for clinical manifestations involving cardiac ECM turnover.[21-23] Lombardi et al. demonstrated that the serum PIIINP level was elevated in hypertrophic cardiomyopathy patients.[3] Querejeta et al. concluded that serum PICP level elevated in patients with heart failure of hypertensive origin.[24] Our previous study also showed that serum PIIINP levels were associated with severity of coronary artery disease and the occurrence of acute rejection in patients receiving heart transplantation.[9,25]

In hypertensive status, arterial hypertension also

**Table 3.** Subgroup analysis of the effect of ACEI/ARB on serum PINP and PIIINP levels among hypertensive patients

|  | ACEI/ARB usage (n = 23) | No ACEI/ARB usage (n = 24) | P value |
|---|---|---|---|
| PINP (μg/L) | 45.8 ± 17.4 | 48.3 ± 24.6 | 0.687 |
| PIIINP (μg/L) | 5.0 ± 2.0 | 4.2 ± 1.5 | 0.128 |

**Table 2.** Serum PINP and PIIINP levels in all study groups

|  | Group 1 (n = 9) | Group 2 (n = 38) | Group 3 (n = 16) | p value[a] | p value (group 1 vs group 2) | p value (group 2 vs group 3) | p value (group 1 vs group 3) |
|---|---|---|---|---|---|---|---|
| PINP (μg/L) | 33.6 ± 13.3 | 50.2 ± 21.6 | 33.0 ± 15.6 | 0.005 | 0.023 | 0.004 | 0.935 |
| PIIINP (μg/L) | 5.9 ± 2.7 | 4.3 ± 1.3 | 3.5 ± 0.7 | 0.001 | 0.005 | 0.1 | < 0.001 |

[a] Value calculated by one-way ANOVA.
PINP = amino-termial propepitde of type I procollagen; PIIINP = amino-terminal propeptide of type III procollagen.

stimulates both myocardial procollagen gene expression and collagen synthesis.[14] The excess deposit of myocardial collagen content results in myocardial stiffness and diastolic dysfunction.[2,26] The association of serum marker of type I collagen and hypertension is also well known in other previous studies,[14,27,28] Querejeta et al. showed serum PICP level correlated well with the extent of left ventricular fibrosis in patients with essential hypertension.[28] Our previous study also revealed the association between serum PINP and diastolic dysfunction in hypertensive patients.[14] In the present study, when comparing with the controlled group, the PINP level was significant elevated in the hypertension alone groups, whereas the PIIINP level was only borderline elevated.

Regarding the influence of diabetes mellitus on cardiac collagen synthesis, Shimizu et al. performed immunohistochemical study of the myocardium in 12 type 2 DM patients and 6 non-diabetic patients.[17] The diabetic patients had significantly higher percentage of type III collagen in the myocardium, while the percentage of type I collagen was similar to that of the controlled group. They hypothesized that hyperglycemia may improve the coronary vascular permeability and collagen accumulation, and assist the afflux of the growth factors to the interstitium. These effects promote fibroblast hyperplasia and speed collagen synthesis. Benazzoug et al. revealed that hyperglycemia enhanced specifically type III collagen synthesis of fibroblasts, not type I collagen.[29] According to these studies, one may expect an increased PIIINP level and a similar PINP level in diabetic patients compared to non-diabetic patients. However, in a recent study, François et al. showed a higher PIIINP but a significant lower PINP level in patients with both hypertension and diabetes mellitus, comparing with the control group.[19] Because the lack of data for hypertensive patients without diabetes mellitus, the individual influence of hypertension or diabetes mellitus on serum PINP and PIIINP levels was not clear. In our study, we found decreased PINP and elevated PIIINP levels in hypertensive patients with diabetes, comparing with the hypertension-alone patients. Furthermore, when we compared the procollagen propeptide levels between the combined hypertension and diabetes group and the control group, the PIIINP level was still

significantly higher in the former group, whereas no significant difference of the PINP level could be detected. Our result showed the influence of diabetes mellitus on elevating the PIIINP and decreasing the PINP level in hypertensive patients. However, there were no normotensive patients with diabetes mellitus in this study. Therefore, we cannot know the influence of diabetes mellitus on collagen turnover in normotensive patients. It depends on further larger-scale clinical trials in the future.

The reason for the negative influence of diabetes mellitus on the PINP level is unclear. Type I collagen is abundant not only in the myocardium, but also in the protein of bone, which comprises about 85% of the bone matrix.[19] Previous in vitro and animal model studies have shown that hyperglycemia has an osteopenia effect, which decreases the osteoblast amount and decreases type I collagen synthesis.[30,31] This may be the reason for decreased PINP level in diabetes patients. However, the evidence of the mechanism is still limited, and further studies are needed to prove the hypothesis. According to the result of this study, we supposed that PINP is not an ideal marker to monitor the cardiac fibrosis extent in hypertensive patients who also have diabetes mellitus. On the contrary, PIIINP is more suitable for cardiac fibrosis evaluation in this subgroup.

Our study has limitations. First, we did not perform endomyocardial biopsy to document fibrotic process. However, it is not ethical to perform endomyocardial biopsy routinely in asymptomatic hypertension and diabetes patients. Besides, in previous study, serum procollagen peptides were reliable in remote monitoring of collagen turnover.[28] Second, the patient number in the present study is small. This may yield to alpha- or beta-type errors in statistical analysis. Studies with larger patient numbers and isolated diabetes patient subgroup are needed for further accurate analysis.

## CONCLUSION

Diabetic status influenced serum PINP and PIIINP levels differently in hypertensive patients. In hypertensive patients with diabetes, serum PIIINP is probably better than serum PINP level for cardiac fibrosis evaluation.

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