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Brief communication (Original)

Pregnancy-associated plasma protein A (PAPP-A) and severity of coronary atherosclerosis assessed by angiographic Gensini score

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Background: Pregnancy-associated plasma protein A (PAPP-A) is a potentially proatherosclerotic metalloproteinase, which has been shown to be abundantly expressed in ruptured unstable plaques. However, changes of the PAPP-A blood levels in patients with coronary artery disease (CAD) according to the Gensini score is unknown in Turkish population.
Objective: We hypothesized that pregnancy-associated plasma protein A (PAPP-A) levels might be important in determining the degree of CAD severity and extent according to its relationship with inflammation in the partly unstable plaque area and with proliferative stimulation overall.
Methods: Our study population included 145 consecutive patients, who underwent elective diagnostic coronary angiography because of CAD symptoms such as chest pain or shortness of breath. The severity and the extent of CAD were evaluated using the Gensini score. Plasma PAPP-A concentrations were determined by ELISA.
Results: Among the 145 study patients (mean age 57 ± 10 years; 97 men and 48 women), 80 had mild CAD (Gensini score <20, group 1), 65 had severe CAD (Gensini score ≥ 20, group 2). PAPP-A levels were significantly higher in group 2 than they were in group 1 (2.7 ± 2.7 μg/ml vs. 1.12 ± 1.3 μg/ml, $p < 0.001$, respectively). PAPP-A levels revealed a moderately positive linear correlation with Gensini scores ($r = 0.435$; $p < 0.001$). In addition, there was a weak, but still a positive correlation between PAPP-A levels and age ($r = 0.174$; $p = 0.03$).
Conclusion: There is still a need for large epidemiological studies to better understand the mechanisms and prognostic roles of both PAPP-A and insulin-like growth factor 1 in asymptomatic subjects and in subjects with well-documented CAD. If the results were promising, the measurement of PAPP-A may become a clinically important tool for risk stratification in patients with chest pain.

Keywords: Atherosclerosis, coronary artery disease, Gensini score, PAPP-A, plasma protein A, pregnancy-associated

Pregnancy-associated plasma protein A (PAPP-A) is a zinc-binding metalloproteinase that is shown to be abundantly expressed in ruptured unstable plaques [1]. It indirectly activates insulin-like growth factor-I (IGF-I), which is a potent mitogen and chemotactic agent for vascular smooth muscle cells through cleaving insulin like growth factor binding protein-4, which releases IGF-1 [2]. Inhibition of IGF-I signaling has shown to delay atherosclerosis [3, 5]. Therefore, PAPP-A appears to modulate growth in local proliferative responses; thus, it indirectly promotes atherosclerosis by increasing the activity of IGF-I.

Although a previous study [1] reported that PAPP-A is expressed in unstable coronary artery plaques, but not in stable coronary artery plaques, Cosin-Sales et al. [6] shown that PAPP-A was associated with angiographic plaque complexity in patients with chronic stable angina pectoris.

In fact, the clinical stability of the patients may not necessarily be an indication of the plaque stability. A study of morphological characteristics of atherosclerotic plaques in chronic stable angina patients revealed that a sizable proportion of these plaques

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show inflammatory features and thrombosis because they were commonly seen in unstable plaques [7]. Moreover, a recent intravascular ultrasound study has shown the presence of coronary plaque rupture in chronic stable angina patients [8]. Overall, it may be assumed that inflammatory processes are involved in every stage of atherosclerosis and they sometimes cycle in and out of one of these clinically defined phases: asymptomatic, stable angina, progressive angina, and acute coronary syndrome.

In the light of this background information, we hypothesized that the PAPP-A levels might have an importance in determining coronary artery disease (CAD) severity and extension according to its relation with inflammation in the partly unstable plaque area and with proliferative stimulation overall. This study in which we have evaluated the PAPP-A levels by classifying the coronary artery patients according to the Gensini score is the first study of its type to be conducted in Turkey.

Material and methods

Study population

Our study population included 145 consecutive patients, who underwent elective diagnostic coronary angiography because of symptoms suggestive of coronary artery disease (CAD) such as chest pain or shortness of breath. None of the patients had acute coronary syndrome or unstable angina pectoris. Diabetes was considered to be present if a person was diagnosed with diabetes according to World Health Organization (WHO) criteria [9]. All subjects were evaluated with a detailed questionnaire and physical examination. The questionnaire provided information about risk factors such as smoking, past medical and family history of coronary artery disease, presence and duration of diabetes mellitus (DM), hypertension, and medical treatment. Patients with ongoing systemic or cardiac inflammatory processes, liver or renal failure, and neoplastic disease were excluded from the study.

The study protocol was approved by our ethics committee and informed consent was obtained from all of the patients.

Assessment of coronary atherosclerosis by angiography

Selective coronary cineangiography was performed with a femoral approach using Judkin's technique. Each of the angiographic series was visually assessed for the presence or absence of stenosis according to a consensus of two experienced angiographers who had no knowledge of the identity and information of the patients. Differences in interpretation were resolved by consensus. Degree of stenosis was defined as the greatest percentage reduction of luminal diameter in any view compared with the nearest normal segment (percent diameter stenosis) and was determined by using the calliper technique [10]. The severity and the extent of coronary artery disease were evaluated using the Gensini score [11]. The Gensini score was computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its topographical importance. Reduction in the lumen diameter and the roentgenographic appearance of concentric lesions as well as eccentric plaques were evaluated (reductions of 25%, 50%, 75%, 90%, 99%, and complete occlusion were assigned Gensini scores of 1, 2, 4, 8, 16, and 32, respectively). Each principal vascular segment was assigned a multiplier in accordance with the functional significance of the myocardial area supplied by that segment: the left main coronary artery, $\times 5$; the proximal segment of left anterior descending coronary artery (LAD), $\times 2.5$; the proximal segment of the circumflex artery, $\times 2.5$; the mid segment of the LAD, $\times 1.5$; the right coronary artery, the distal segment of the LAD, the posterolateral artery, and the obtuse marginal artery, $\times 1$; and others, $\times 0.5$.

The patients were divided into two groups according to their Gensini scores. Group 1 is mild coronary artery disease with Gensini score <20 and group 2 is severe coronary artery disease with Gensini score ≥ 20 [12].

Blood sample collection

Two tubes of fasting blood samples were drawn before angiography was performed. The first one was used for the determination of biochemical risk factors including glucose, lipid profile, and apolipoprotein analyses. The second one was chilled to –80°C after separation of serum by centrifugation at 4000 rpm for 5 minutes to use in PAPP-A level measurement.

Laboratory measurements

Glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, lipoprotein (Lp)(a), apolipoprotein (Apo) A1, and Apo B were determined by routine methods. PAPP-A levels were determined

using commercial sandwich-ELISA kit (DRG International, Mountainside, NJ, USA; Catalog number: EIA-2397) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were conducted by utilizing the SPSS for Windows (version 11.5, Chicago, Ill, USA). The chi-square test was used to test for differences in categorical variables, such as gender, hypertension, smoking status, and family history. Spearman Rho's and Pearson tests were used for correlation analyses. Multiple linear regression analysis was performed in order to relate Gensini scores to independent risk factors. A value of $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

Among the 145 study patients (mean age 57 ± 10 years; 97 men and 48 women), 80 had mild CAD (Gensini score <20, group 1), 65 had severe CAD (Gensini score ≥ 20, group 2). There were no significant differences in age and coronary risk factors such as hypertension, family history, smoking, type-2 DM, and lipid profile except for triglycerides. Men were in the majority in both groups, but male gender was significantly more in group 2 than it was in group 1 (83% vs. 53%; $p < 0.001$ respectively) as show in **Table 1**.

In group 2, presence of DM, levels of triglycerides and glucose were significantly higher than they were in group 1.

PAPP-A levels were significantly higher in group 2 than they were in group 1 (2.7 ± 2.7 μg/ml vs. 1.12 ± 1.3 μg/ml, $p < 0.001$, respectively).

Correlation analysis

PAPP-A levels revealed a moderately positive linear correlation with Gensini scores ($r = 0.435$; $p < 0.001$). In addition, there was a weak but still a positive correlation between PAPP-A levels and age ($r = 0.174$; $p = 0.03$).

Determinants of the Gensini score

In multivariate logistic regression analysis including 12 variables for Gensini score ≥ 20 as dependent parameters, male gender ($p < 0.001$), presence of hypertension ($p = 0.03$), glucose ($p = 0.02$) and PAPP-A levels ($p = 0.01$) emerged as significant independent determinants (**Table 2**).

Table 1. Demographic characteristics of the groups

	Group 1 (Gensini score <20) n = 80	Group 2 (Gensini score ≥ 20) n = 65	*p*
PAPP-A (μg/ml)	1.12 ± 1.34	2.7 ± 2.7	<0.001
Age (years)	55.3 ± 8.9	58.5 ± 10.4	NS
Gender (male/female)	43/37	54/11	<0.001
Type-2 DM (%)	12.5	24.6	NS
Hypertension (%)	50	61.5	NS
Smoking (%)	38.7	40	NS
Glucose (mg/dl)	105.7 ± 33.8	125.6 ± 58.2	<0.011
HDL-C (mg/dl)	42.4 ± 13.1	39.4 ± 10.5	NS
LDL-C (mg/dl)	107 ± 34.5	114.3 ± 43.7	NS
Total Cholesterol (mg/dl)	173.7 ± 43.3	183 ± 52	NS
Triglycerides (mg/dl)	119.4 ± 67.7	144.6 ± 81.2	0.004
Lipoprotein (a) (mg/dl)	22.2 ± 22.3	25.3 ± 29.7	NS
Apo-A1 (mg/dl)	132.7 ± 24.4	128.9 ± 29.2	NS
Apo-B (mg/dl)	93.1 ± 27.9	97.7 ± 27.6	NS
Duration of type-2 DM (years)	0.8 ± 3.6	1.4 ± 3.2	NS

PAPP-A = pregnancy-associated plasma protein A, DM = diabetes mellitus, HDL-C = high density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol, Apo = apolipoprotein

Table 2. Multivariate logistic regression analysis of groups

	Wald–chi square	Odds ratio	95% CI	*p*
Age	0.9	1.02	0.975–1.078	0.3
Male gender	13.3	8.9	2.754–29.141	<0.001
Family history of CAD	3.6	2.4	0.971–6.139	0.6
Smoking	0.5	0.5	0.547–3.536	0.5
Hypertension	4.6	2.7	1.098–6.979	0.03
Type-2 DM	0.4	0.6	0.157–2.484	0.5
PAPP-A	11.7	1.6	1.214–2.038	0.01
HDL-C	1.1	1.1	0.939–1.228	0.3
LDL-C	1.4	1.1	0.953–1.216	0.2
Triglycerides	1.7	1.0	0.992–1.044	0.2
Total cholesterol	1.2	0.9	0.826–1.055	0.3
Glucose	5.7	1.0	1.003–1.031	0.02

CAD = coronary artery disease, DM = diabetes mellitus, PAPP-A = pregnancy-associated plasma protein A

Discussion

This study demonstrates that circulating PAPP-A levels are associated with coronary artery disease severity and extension in patients with angiographically documented severe coronary artery disease, but without acute coronary syndrome.

Despite the pioneering work of Bayes et al. [1], which proposed the PAPP-A was highly correlated with the unstable plaques, but not with the stable plaques, this study along with several others might have an important role in pointing out that PAPP-A may not be the only marker of vulnerable plaques. Furthermore, other studies suggested that elevated PAPP-A levels were not only limited to acute coronary syndrome, but also have a substantial link to the earlier stages of atherosclerotic lesions [13-16].

In conjunction to this, in another study, Beaudeux et al. [17] were able to show that the elevated levels of PAPP-A were related to premature development of atherosclerosis in the carotid artery in hyperlipidemic asymptomatic patients. Similarly study [18] also reported that PAPP-A was not only related to plaque instability, but also served as a marker of total atherosclerotic burden in asymptomatic patients with hyperlipidemia.

In our study population, LDL-C levels were mildly elevated and PAPP-A levels were not correlated with any of the lipid levels. In addition, as was also reported in previous studies, we were not able to correlate PAPP-A with sex, CAD risk factors such as hypertension, DM, or smoking.

In the cohort of Colin-Sales et al. [6], patients with stable angina undergoing coronary angiography had both serum PAPP-A and CRP levels associated with the complexity of the coronary artery disease. In the same study, they were also able to give a cut-off point of 4.5 mIU/L to predict the presence of significant stenosis with a sensitivity of 45% and a specificity of 84%. Our results, resembling Colin-Sales et al. [6], also indicated that increasing serum PAPP-A levels were significantly associated with a high Gensini score (≥ 20), which was indicative of severe coronary artery disease. However, we were not able to determine such a cut-off point because of the wide range of distribution of the serum PAPP-A values.

Although there is still a debate regarding whether PAPP-A is related to only unstable plaques, but not with stable plaques, our results support the study by Colin-Sales et al. [6]. We think that these conflicting results might be the result of the dual role of PAPP-A in both plaque growth and in disruption through different effects of IGF-1.

In other words, while IGF-1 induced chemotaxis and macrophage activation may have an effect on plaque disruption, IGF-1 induced LDL cholesterol uptake by macrophages and mitogenic induction on vascular smooth muscle cell may have an effect on plaque growth. Moreover, even in patients without acute coronary syndrome, there may be still some high lipid core vulnerable plaques [4] or complex lesions that carry both modalities of stable and unstable plaques.

To date, the exact pathogenetic mechanism of PAPP-A in plaque growth and disruption remains unclear. Collecting evidence from different studies will be helpful in understanding the mechanism of PAPP-A and IGF-1 in atherosclerosis.

A recent report [19] evaluated PAPP-A levels in 3782 patients with non-NSTE-ACS (non-ST-segment elevation acute coronary syndrome). They concluded that PAPP-A was independently associated with the short- and long-term risk of cardiovascular death and recurrent ischemic events in patients with NSTE-ACS along with clinical predictors and troponin I. Furthermore, they suggested that PAPP-A might be a prognostic marker for recurrent ischemia and cardiovascular death in patients with ACS. In addition, two similar studies reported that increased PAPP-A levels were an independent predictor of future ischemic events in patients presenting to the emergency department with suspected acute coronary syndrome [15, 20]. The findings we have obtained in this study confirm the findings of these previous studies.

The size of our study population was limited. The presence of type-2 DM was higher in the severe CAD group than it was in the mild CAD group. In one study, it has been reported that serum PAPP-A levels increased in type-2 DM [21]. Therefore, there is a debate regarding whether or not more frequent existence of type-2 DM in severe CAD group compared to mild CAD group had an influence on the increased serum levels of PAPP-A in severe CAD group. By contrast, as in several (but not all) previous studies, in our study, serum CRP levels failed to correlate with PAPP-A levels. Moreover, we did not measure highly sensitive CRP, whereas all the other studies did measure highly sensitive CRP.

In conclusion, there is still a need for large epidemiological studies to better understand the mechanisms and prognostic roles of both PAPP-A and IGF-1 in asymptomatic subjects and in subjects with a well-documented CAD. If the results were promising, the measurement of PAPP-A may become a clinically important tool for risk stratification in patients with chest pain. However, this requires further investigation.

The authors have no conflicts of interest to declare.

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