Journal of Cardiology 61 (2013) 128–131

Contents lists available at SciVerse ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Reference interval of pregnancy-associated plasma protein-A in healthy men and non-pregnant women

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ARTICLE INFO

Article history:
Received 24 July 2012
Received in revised form 31 August 2012
Accepted 10 September 2012
Available online 16 November 2012

Keywords:
Acute coronary syndrome
PAPP-A
Partition
Reference interval
Smoking

ABSTRACT

Objective: The serum pregnancy-associated plasma protein-A (PAPP-A) concentration is a predictor of ischemic cardiac events and renal impairment. However, the reference interval of PAPP-A has not been determined. This study determined the reference interval of PAPP-A in men and non-pregnant women.
Methods: The study enrolled 126 apparently healthy individuals (52 males and 74 females). The mean age of the men and women was 34.7 (range 20–66) years and 34.6 (range 18–65) years, respectively. Serum PAPP-A concentrations were determined using an ultrasensitive enzyme-linked immunoassay kit. Reference intervals were calculated using the bootstrap method.
Results: The results for three subjects were outliers, so the reference interval of PAPP-A was calculated using the data for 123 subjects. PAPP-A was undetectable in 26 subjects. The reference interval of PAPP-A for men and women (with the 90% confidence interval) was <22.9 ng/mL (19.7–23.3) and <33.6 ng/mL (25.2–36.7), respectively. In male subjects, serum PAPP-A levels of smokers [3.10 (UD, 7.30) ng/mL] were significantly lower than that of non-smokers [11.00 (UD, 24.4) ng/mL] ($p < 0.001$) and there was a positive correlation between serum PAPP-A levels and subjects' age ($r = 0.439$; $p < 0.001$).
Conclusions: The reference interval of PAPP-A differed for men and non-pregnant women. In clinical practice, <22.9 ng/mL for men and <33.6 ng/mL for non-pregnant women may be used as reference intervals for PAPP-A.

Introduction

Pregnancy-associated plasma protein-A (PAPP-A) is a member of the insulin-like growth factor (IGF) axis [1]. This axis includes growth hormone (GH), IGF-1 and -2, six IGF-binding proteins (IGFBP-1–6), and IGFBP proteases. Normally, IGFs are bound to IGFBPs, which act as IGF carriers, but also modulate IGF availability and activity [2]. PAPP-A is a protease (pappalysin-1, EC 3.4.24.79) of IGFBPs and its primary substrate is IGFBP-4, although it also cleaves IGFBP-2 and IGFBP-5 [1,3,4]. The proteolytic activity of PAPP-A against IGBP-4 is IGF-dependent and it cleaves IGFBP-4 when it is complexed with IGF. PAPP-A cleaves IGFBPs (IGFBP 2, 4, 5) and increases the local concentrations of IGFs, which are involved in the regulation of growth, proliferation, and differentiation of various cell types.

The serum mass concentrations of PAPP-A in the first and second trimesters are used to screen for genetic anomalies, particularly Down's syndrome [5]. In addition to pregnancy, the clinical value of PAPP-A, particularly in men and non-pregnant women, continues to grow as new data become available. PAPP-A is potentially proatherosclerotic and high mass concentrations have been reported in acute coronary syndromes (ACSs), asthma, and renal impairment [6–9]. The serum PAPP-A mass concentration is an independent predictor of cardiac ischemia in patients who present with suspected myocardial infarction, but remain troponin-negative [10].

In ACS patients, elevated PAPP-A originates from atherosclerotic plaques [11]. Bayes-Genis et al. used immunohistochemical staining with antihuman PAPP-A antibodies and demonstrated that PAPP-A was abundantly expressed in the eroded and ruptured coronary atherosclerotic plaques [9]. Additionally, they showed that the cells that synthesize and secrete PAPP-A within the atherosclerotic plaque are likely vascular endothelial and smooth muscle cells [9].

Although great progress has been made in determining the structure and function of PAPP-A, some variables need to be evaluated in clinical practice, including the reference interval and

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0914-5087/$ – see front matter
http://dx.doi.org/10.1016/j.jjcc.2012.09.007

diagnostic sensitivity and specificity, particularly for cardiovascular events. Here, for the first time, we investigated the reference interval of PAPP-A in men and non-pregnant women.

Materials and methods

The study was conducted at the Acibadem Labmed Clinical Laboratories, Istanbul, Turkey. The study protocol was approved by the Ethics Committee of Acibadem University and all subjects gave written informed consent before participating.

We enrolled 126 apparently healthy individuals (52 men and 74 women) to investigate the reference interval of PAPP-A. The mean age of the men and women was 34.7 (range 20–66) years and 34.6 (range 18–65) years, respectively. The subjects considered themselves to be healthy and had no history of heart failure, hypertension, hyperlipidemia, renal impairment, diabetes, major trauma, or surgery. The characteristics of the study population are shown in Table 1. No subjects were taking any prescribed drugs or had been hospitalized recently. In addition, the female subjects were confirmed to be not pregnant.

With the subjects in a sitting position, venous blood was collected from an antecubital vein. Samples were transported to the laboratory under identical conditions of temperature and elapsed time. After clotting, all samples were centrifuged at $1500 \times g$ and the serum was stored at $-80\,^{\circ}C$ until analysis. At the end of the study, all samples were analyzed on the same day in one run using a PAPP-A enzyme-linked immunoassay (ELISA) kit (ultrasensitive ELISA kit, DRG Instruments, Marburg, Germany). According to the manufacturer, the within-run coefficient of variation (CV) was 6.86% and the detection limit of the method was 0.023 ng/mL.

Statistical analysis

We examined the data for outlying values visually and statistically. We plotted the data distribution and then excluded outliers that were not part of the reference distribution. We used the Kolmogorov–Smirnov test to evaluate the normality of data. The Mann–Whitney *U* test was used to evaluate the significance of differences between serum PAPP-A levels of males and females, smoking and non-smoking subjects, and pre-menopause and post-menopause women. Correlations between variables were assessed using Spearman correlation analysis. Values of $p < 0.05$ were considered as statistically significant.

To determine the presence of possible subclasses in the entire dataset, we calculated the *z* and *z*-critical values, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [12]. Finally, we used a non-parametric bootstrap approach to calculate the reference interval of PAPP-A.

Table 1
Subject characteristics.

Characteristics	Female (*n*: 73)	Male (*n*: 50)
Age, year, mean (range)	34.8 (18–65)	34.3 (20–66)
Menopause (*n*)	13	
Body mass index, mean (range)	23.5 (18.2–38.9)	25.9 (19.9–34.4)
Smoking (*n*)	10	11
Hypertension	–	–
Diabetes	–	–
Hyperlipidemia	–	–
Heart failure	–	–
Renal impairment	–	–
Major trauma	–	–
Surgery within one year	–	–
Prescribed drugs	–	–

The pregnancy-associated plasma protein-A levels of three (two men and one woman) subjects were extreme values and so were removed from the study. Thus, the data of remaining 123 subjects were used.

Table 2
PAPP-A levels in study population.

Variables (*n*)	Sub-variables (*n*)	PAPP-A (ng/mL); median (min., max.)	*p*
Male (50) Female (73)		6.85 (UD, 24.40) 3.40 (UD, 36.70)	<0.011
Male	Smoking (11) Non-smoking	3.10 (UD, 7.30) 11.00 (UD, 24.40)	<0.001
Female	Smoking (10) Non-smoking	3.00 (UD, 36.70) 4.10 (UD, 36.70)	<0.397
	Pre-menopause Post-menopause (13)	2.60 (UD, 36.70) 8.50 (UD, 21.60)	<0.137

UD, undetectable.

Results

PAPP-A levels

As shown in Table 2, the median serum PAPP-A levels in male subjects [6.85 (undetectable (UD), 24.40) ng/mL] were significantly higher than that of female subjects [3.40 (UD, 36.7) ng/mL] ($p < 0.011$). In male subjects, serum PAPP-A levels were significantly lower in smokers [3.10 (UD, 7.30) ng/mL] than in non-smokers [11.00 (UD, 24.4) ng/mL] ($p < 0.001$). Similarly, in female subjects serum PAPP-A levels were lower in smokers [3.00 (UD, 36.70) ng/mL] than in non-smokers [4.10 (UD, 36.70) ng/mL]. However, this difference was not statistically significant ($p > 0.05$). Additionally, in female subjects the PAPP-A levels in pre-menopause women [2.6 (UD, 36.7) ng/mL], were lower than the post-menopause women [8.5 (UD, 21.60) ng/mL], however this difference was not statistically significant, either ($p > 0.05$).

Correlation analysis

In male subjects, we found a statistically significant correlation between serum PAPP-A levels and subjects' age ($r = 0.439$; $p < 0.001$); however, in female subjects it was not statistically significant ($r = 0.103$; $p < 0.383$). The correlation between serum PAPP-A levels and body mass index (BMI) in both male and female subjects was not statistically significant ($p > 0.05$).

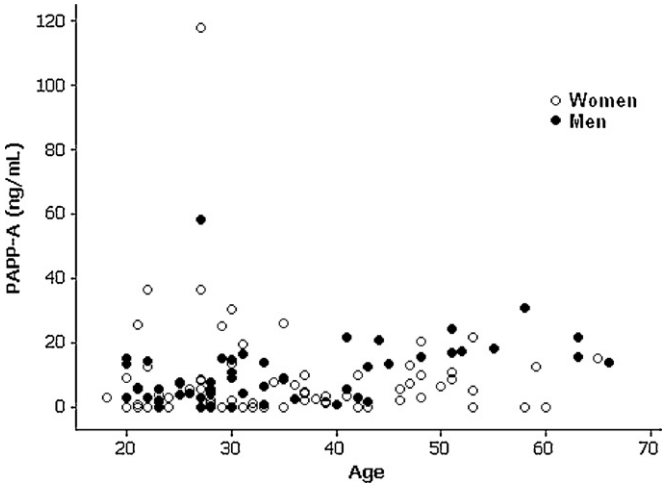

Fig. 1. Serum pregnancy-associated plasma protein-A (PAPP-A) levels of all subjects as one group.

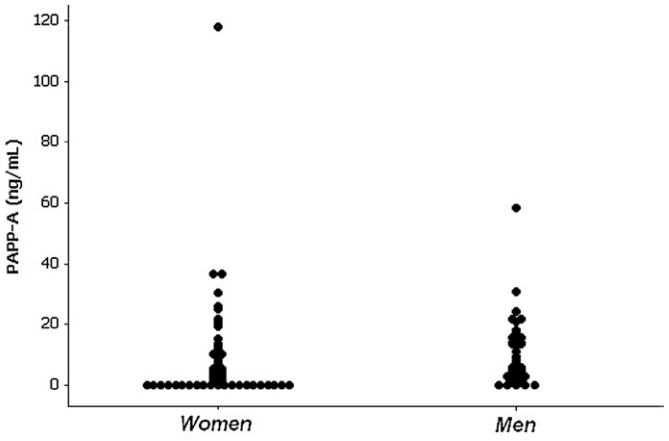

Fig. 2. Serum pregnancy-associated plasma protein-A (PAPP-A) levels of women and men as separate groups.

Reference intervals

Fig. 1 shows the serum PAPP-A levels of all 126 subjects. We also drew a graph to determine whether the data for men and women clustered separately (Fig. 2). As Figs. 1 and 2 show, the PAPP-A levels of three (two men and one woman) subjects were extreme values and so were removed from the study. The *z*-value of the male and female groups (3.08) exceeded *z*-critical (2.16). Therefore, we separated the data for men and women.

PAPP-A was not measurable in 5 men and 21 women; consequently we determined only the upper reference limit using the nonparametric bootstrap method. The calculated upper reference limits and confidence intervals for PAPP-A for men and women were 22.9 ng/mL (19.7–23.3) and 33.6 ng/mL (25.2–36.7), respectively. Therefore, the PAPP-A reference intervals in men and in non-pregnant women are <22.9 ng/mL and <33.6 ng/mL, respectively.

Discussion

Many papers on the relationship between PAPP-A and ACS have been published [9–11], but a population-based reference interval has not been determined. In this study, for the first time, we have determined a population-based reference interval of PAPP-A in men and non-pregnant women.

Previously, we found that the index of individuality (*II*) of PAPP-A was 0.95 [13]. If $II < 0.48$ for a test, the population-based reference values will be of little utility, while if $II > 1.4$, the population-based reference values are significant [14]. For PAPP-A, $II = 0.95$, meaning that the population-based reference values for PAPP-A have moderate utility. This means that partitioning may be needed in reference value studies of PAPP-A. In this study, the *z*-values calculated for the data for men and women were greater than *z*-critical. Accordingly, these results suggest that partitioning is necessary and that one reference interval may not be useful clinically for both men and women.

This study included 126 subjects, which is adequate for non-parametric calculation of the reference interval. Due to partitioning, however, each group had <120 subjects. Consequently, we preferred a bootstrap method over a non-parametric approach. In the bootstrap method, a dataset is resampled randomly with replacement, i.e. when an item is sampled it is replaced immediately, multiple times (as many as 1000–10,000 or more times) and statistical calculations, such as the reference and confidence intervals in our study, are performed using this huge data collection [15]. The bootstrap method is preferred for reference interval calculation when the number of subjects is <120. With few subjects, it gives a more realistic confidence interval than the non-parametric approach.

The reference interval for men was <22.9 ng/mL and for women was <33.6 ng/mL. Interestingly, as shown in Table 2, the median of serum PAPP-A levels in men (6.85 ng/mL) was higher than non-pregnant women (3.40 ng/mL) ($p < 0.01$). As shown in Fig. 2, despite high median level, the dispersion of male data was narrower than female subjects and consequently the upper limit of women reference interval was higher than men.

Various molecules such as pentraxin 3 [16], N-terminal pro-B-type natriuretic peptide [17], urinary liver-type fatty acid binding protein [18], and PAPP-A are being proposed as biomarkers for ACS. However, high level of PAPP-A seems to be an independent risk factor for cardiovascular event in patients with ACS [19]. Lobbes et al. have studied 120 patients (87 males and 33 females) using an ultrasensitive ELISA method (DRG) and found that PAPP-A is elevated in acute myocardial infarction (25.5 ng/mL) and stable (32.9 ng/mL) and unstable (52.9 ng/mL) angina pectoris. The data in parenthesis are the mean differences of patient data from control group [20]. Heeschen et al. studied 644 patients with acute chest pain. They found that elevated PAPP-A levels [>12.6 mIU/L (56.7 ng/mL; 1 mIU = 4.5 μg)] indicated an increased risk for cardiovascular events [19]. In another study, Heider et al. found elevated levels of PAPP-A in unstable versus stable plaques in patients (0.10 ± 0.06 μg/mL vs. 0.07 ± 0.04 μg/mL; i.e. 100 ± 60 ng/mL vs. 70 ± 40 ng/mL) with carotid artery stenosis and correlation with cap thickness [21]. Based on these findings, we suggest that our reference interval data may be used in clinical practice for healthy men and non-pregnant women.

In our study, we have shown that PAPP-A levels were lower in smokers than in non-smokers. In pregnant women smoking is associated with a reduction in birth size. It has been shown that smoking during pregnancy decreases serum PAPP-A levels [22,23]. Smoking is a major risk factor for cardiovascular disease and the high level of PAPP-A in cardiovascular events has been reported in various papers. The inverse relation of smoking and PAPP-A needs to be confirmed in large population studies.

Another important finding of the present study is the positive correlation between age and PAPP-A levels in male subjects (Fig. 3). Recent studies using genetically engineered mice indicate a newly recognized role for PAPP-A in aging and in the development of age-related disease [24]. Conover and co-workers have shown that PAPP-A knock-out mice live 30–40% longer than wild-type littermates [25]. The role of PAPP-A in aging and age-related disease needs to be investigated.

There are some limitations of the present study. In the literature different results of PAPP-A in various clinical situations have

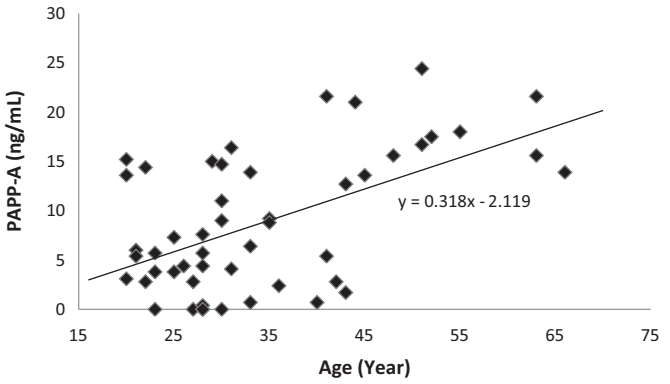

Fig. 3. Correlation between serum pregnancy-associated plasma protein-A (PAPP-A) levels and age in male subjects.

been reported. The numerical value of PAPP-A is closely related to the types of measurement method. Commonly ELISA, ultrasensitive ELISA, and electrochemiluminescence are used to determine serum PAPP-A levels. Unfortunately, there has not been any standardization among these methods, particularly for low PAPP-A levels in men and non-pregnant women. We have used an ultrasensitive ELISA method (DRG). Thus, our reference interval should be interpreted accordingly. An interesting finding of this study is the low level of PAPP-A in smokers. The number of smokers in both male and female groups may be adequate for statistical comparison, but not adequate for determination of reference intervals. For that reason, the reference interval of PAPP-A in the smoking population should be determined separately.

Taken together, in the light of the literature we conclude that the upper reference limit of PAPP-A determined in our laboratory (<22.9 ng/mL for men and <33.6 ng/mL for women), can be used in clinical practice, except in children and pregnant women.

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