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Plasma levels of atrial and brain natriuretic peptides in apparently healthy subjects: Effects of sex, age, and hemoglobin concentration

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ABSTRACT

Background: To examine whether the use of one value of natriuretic peptides to define "normal" is appropriate in all individuals, and to assess the influence of sex, age, and other variables on atrial and brain natriuretic peptides (ANP, BNP) levels.

Methods and results: A total of 1375 apparently healthy people (women:155, men:1220), aged 18–70 years were enrolled. Both ANP and BNP levels were higher in women than in men (ANP: 12.50 ± 6.82 pg/mL vs 8.18 ± 4.19 pg/mL; BNP: 9.85 ± 7.63 pg/mL vs 7.03 ± 6.97 pg/mL). The subjects were divided into three age groups: group I, 18–30 years; group II, 30–50 years; group III, 50–70 years. First, the influence of age on ANP and BNP levels was examined. In women, both ANP and BNP levels were higher in groups II and III than those in group I. In men, ANP and BNP levels increased with age. Second, sex differences in ANP and BNP levels due to age were examined. ANP level was higher in women than that in men in all age groups. BNP level was higher in women than that in men in groups I and II. Multivariate analysis indicated that both ANP and BNP levels were influenced by age, hemoglobin level, and platelet counts.

Conclusion: Because ANP and BNP levels in healthy subjects are influenced by sex, age, and hemoglobin levels, the use of a single value to define "normal" in all individuals is not appropriate.

1. Introduction

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels increase in congestive heart failure [1,2], and their plasma levels have been used to estimate the severity and prognosis of heart failure [3–6]. In addition, ANP and BNP levels are commonly used to screen the general population for heart disease [7,8]. Therefore, it is critical to determine the cut-off levels of normal plasma ANP and BNP. Even among apparently healthy subjects with normal BNP levels, the incidence of heart failure is higher among those with levels in the highest third of the range [9]. It has also been reported that the upper normal level of BNP is too strict for diagnosing significant organic heart disease, and that a higher cut-off level would be preferable and more realistic in clinical practice [10]. The effects of sex, age and other variables on normal ANP and BNP levels remain to be determined [9,11–14]. Furthermore, whether one "normal" value of ANP and BNP is appropriate for all subjects considering the huge variability in individual characteristics seems to be an important problem in the clinical setting.

Therefore, we aimed to examine the effects of sex, age, and other variables on ANP and BNP levels in apparently healthy subjects, and to examine whether the use of one normal value of ANP and BNP is appropriate in all subjects.

2. Methods

2.1. Study subjects

This study was approved by the Human Investigations Committee of Uwajima City Hospital (no. 1510-85). The participants comprised 1375 apparently healthy people aged 18–70 years who were employed at two companies: the Matsuyama branch of the Shikoku Electric Power Company and the Daio Paper Corporation. All subjects met the following criteria: 1) never received treatment for cardiovascular diseases; 2) no atrial fibrillation, abnormal Q wave, or negative T wave shown by electrocardiography; 3) plasma creatinine level <1.4 mg/dL and glycated hemoglobin A1c (HbA1c) <7.0%; 4) body mass index (BMI) <30. A consistent inverse relationship between obesity and circulating ANP or BNP has been reported [15–17]. To remove the effect of this relationship on the results, we excluded subjects with a BMI >30.

Blood sampling was performed in 2003 as a part of regular medical examinations at the companies. In addition to the measurement of the number of red blood cells (RBC),

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hemoglobin concentration (Hb), platelet (PLT) count, and the levels of creatinine (Cr), HbA1c, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), we also measured plasma levels of ANP and BNP. In addition, to examine the influence of age on ANP, BNP, and other variables, the subjects were divided into three groups according to age: group I, 18–30 years; group II, 30–50 years; group III, 50–70 years.

2.2. Natriuretic peptide assays

Plasma ANP concentrations were measured using a Shiono RIA assay kit (Shionogi Co. Ltd., Osaka, Japan), and plasma BNP concentrations were measured with a specific commercial immunoradiometric assay kit for human BNP (Shionogi Co. Ltd., Osaka, Japan). The fasting subjects underwent phlebotomy in a sitting position, typically at 8–10 AM. Samples were immediately transferred to chilled tubes containing ethylenediaminetetraacetic acid, centrifuged, and then the plasma was frozen at −70 °C. The lower limits of detection for the ANP and BNP assays were <0.1 pg/mL.

2.3. Statistical analysis

All values are expressed as the mean ± standard deviation. Statistical analysis was performed using JMP 10 software (SAS, Cary, NC, USA). For continuous variables, comparisons were made between the two groups using with Student's *t*-test. Correlations between two parameters were assessed using simple linear regression. Univariate and multivariate linear regression analyses were used to assess the effect of clinical parameters on ANP and BNP. Multivariate regression analysis was performed on all factors that were significantly associated with natriuretic peptides by univariate analysis. A p-value <0.05 was considered statistically significant.

3. Results

3.1. ANP, BNP, and other variables in women and men

Table 1 shows the number of subjects, their mean age, mean values of ANP, BNP, and other variables in women and men. The levels of ANP and BNP were higher in women than those in men. RBC, Hb, Cr, and TG values were lower in women than those in men. PLT and HDL-C levels were higher in women than those in men. TC and HbA1c showed no significant difference between women and men.

3.2. Distribution of plasma ANP and BNP levels

Fig. 1 shows the distribution of plasma concentrations of ANP (1-A) and BNP (1-B). The maximal levels of ANP were 5–7 pg/mL, as shown in Fig. 1-A. There was only one subject with ANP level <3 pg/mL (2.9 pg/mL). The maximal BNP levels were 3–5 pg/mL, as shown in Fig. 1-B. There were 21 subjects whose BNP levels were below the detection limit. Very few subjects had ANP or BNP levels >30 pg/mL.

3.3. Influence of age on ANP, BNP, and other variables

Table 2 shows the data regarding the numbers of subjects with each ANP and BNP level and of all variables in each group. First, we examined the influence of age on each variable in women and men. In women, as shown in Fig. 2-A and -B, the ANP and BNP levels were higher in group II than in those in group I. There were no significant differences in ANP and BNP levels between groups II and III. In men, as shown in Fig. 2-C and -D, both ANP and BNP levels significantly increased with increasing age.

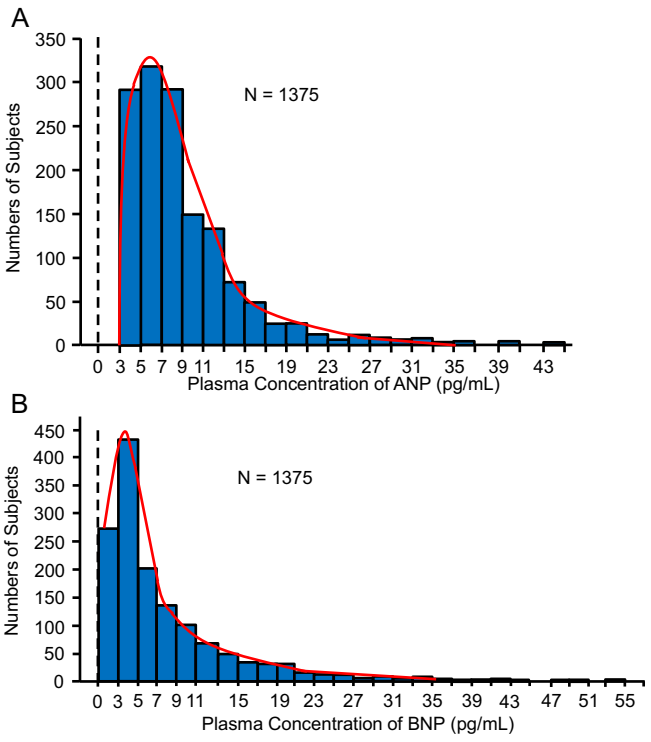

Fig. 1. The distribution of the plasma concentrations of ANP (1-A) and BNP (1-B) among 1375 apparently healthy subjects. Blue bars indicate the number of subjects with each concentration of ANP or BNP. The red line indicates the trend. ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide.

Among the other variables, as shown in Table 2, RBC, Hb, PLT, or HDL-C did not differ among the three groups in women. Cr level was the lowest in group I, and the TC and HbA1c values were the highest in group III. TG significantly increased with age. In men, as shown in Table 2, RBC and Hb significantly decreased, and HbA1c increased with increasing age. Cr, TC, and TG levels were the lowest in group I.

Second, we compared all variables between women and men in each group. ANP levels were higher in women than those in men in all groups. The BNP was higher in women than that in men in groups I and II, but there was no significant difference in the BNP levels of men and women in group III. From the other variables, RBC, Hb, Cr, and TG values were lower in women than in men, and HDL-C was higher in women than in men in all groups.

3.4. Determinants of plasma levels of ANP and BNP

Table 3 indicates the determinants of plasma levels of ANP (A) and BNP (B). In univariate analysis, the level of ANP was significantly correlated with age, RBC, Hb, PLT, TG, and HDL-C levels. In multivariate analysis, the level of ANP was significantly correlated with age, Hb, PLT, and TG values. In univariate analysis, the BNP level was significantly

Table 1
Plasma levels of atrial natriuretic peptide, brain natriuretic peptide and other variables in female and male apparently healthy subjects.

	n	Age (years)	ANP (pg/mL)	BNP (pg/mL)	RBC (10^4/μL)	Hb (g/dL)	PLT (10^4/μL)	Cr (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	HbA1c (%)
Total females	155	34.1	12.5	9.9	444.9	12.8	24.4	0.67	198	69	67	4.9
		13.2	6.8	7.6	30.6	1.3	7.0	0.12	31	36	13	0.6
Total males	1220	38.3	8.2	7.0	496.2	15.4	23.2	0.93	194	127	54	5.1
		11.9	4.2	7.0	37.1	0.8	5.1	0.17	60	93	12	0.8
p value		0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.0245	<0.0001	0.1460	<0.0001	<0.0001	0.0543

Data given as mean (upper number) ± SD (lower number). Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; Cr, creatinine; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin A1c.