Original Article

Circadian Variation of N-terminal Pro-B-type Natriuretic Peptide in a Normal Population

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Background: The N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a well-known hormone used for the clinical diagnosis and evaluation of patients with heart failure. Its half-life is only 60 to 120 minutes may vary depending on the time of day, and its circadian variation is undetermined. The efficacy of NT-proBNP may be affected if there is circadian variation, and normal values may fluctuate depending on the time of day.

Objective: To determine the difference in NT-proBNP levels in healthy volunteers at varying times of the same day.

Materials and Methods: The present study enrolled a total of 30 healthy volunteers who were over 20 years old and had normal physical examination, renal function, liver function, chest radiography and electrocardiography in the year preceding study enrollment. The volunteers did not take any medication, including vitamins or energy drinks, in the week before enrollment. Blood samples for NT-proBNP levels were collected at 8.00 AM, 12.00 noon, 4.00 PM, 8.00 PM and 12.00 midnight on the same day.

Results: The present study enrolled a total of 30 volunteers, most of whom were female (66.6%). Their mean age was 30.30±5.98 years old; mean BMI was 20.34±3.57 kg/m^2; mean serum creatinine was 0.79±0.16 mg/dL; mean serum AST was 21.8±5.4 IU/L; and mean serum ALT was 22.57±6.51 IU/L. The mean levels of NT-proBNP at 8.00 AM, 12.00 noon, 4.00 PM, 8.00 PM and 12.00 midnight were 36.2±29.9 pg/mL, 35.4±30.3 pg/mL, 39.0±40.9 pg/mL, 32.4±36.1 pg/mL and 23.8±20.3 pg/mL, respectively. There was no significant difference in NT-proBNP levels over the various time periods.

Conclusion: The levels of NT-proBNP of healthy volunteers did not show any circadian variation; however, the levels of NT-proBNP tended to be highest at 4.00 PM and lowest at 12.00 midnight.

Keywords: N-terminal pro-B-type natriuretic peptide, NT-proBNP, BNP, Heart failure, Healthy volunteers

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Heart failure is a major public health problem in many countries, including Thailand. Diagnosis of heart failure is made clinically from symptoms and signs[1], and a number of investigations are commonly used to confirm the diagnosis. In some patients, clinical diagnosis is inconclusive and in these cases, natriuretic peptide levels may provide additional diagnostic benefits.

Natriuretic peptide is released from the ventricles in response to increased wall stress, and salt and water retention. The natriuretic peptides used in clinical practice include B-type natriuretic peptide (BNP) or N-terminal pro-B-type natriuretic peptide (NT-proBNP)[2]. BNP and NT-proBNP are used in the diagnosis of heart failure[2] and have high sensitivity, specificity and survival prediction rates at 10 years[3].

Previous studies have shown that various factors affect natriuretic peptide levels including age[4,5], gender[4-6], obesity[7], concurrent medication and renal function[8,9]. The half-life of BNP is only 20 minutes while that of NT-proBNP is 60 to 120 minutes[2], and the levels of BNP may vary over the 24 hours of the day[10]. The purpose of this research was to evaluate the diurnal variations of NT-proBNP in a normal population.

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Materials and Methods

Study population

Healthy volunteers of either sex were eligible for inclusion in the present study if they were over 20 years old. The volunteers were asymptomatic and healthy with normal renal function, liver function, chest x-ray and electrocardiogram (ECG) in the year before enrollment in the present study. Subjects were excluded if they had received any medication including vitamins or any energy drinks that contained caffeine or herbs.

Study protocol

A cross-sectional study was performed. Normal volunteers were enrolled, and baseline characteristics such as age, sex, body weight, and height were recorded.

NT-proBNP was measured by the electrochemiluminescence Immunoassay (ECLIA) technique. The blood

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samples were collected to measure NT-proBNP levels at 8.00 AM, 12.00 noon, 4.00 PM, 8.00 PM and 12.00 midnight on the same day to determine whether there were differences in NT-proBNP levels in this normal population depending on the time of day.

The protocol of this research was reviewed and approved by the ethics committee of Rajavithi Hospital (No. 106/2560).

Statistical analysis

The estimated sample size was based on a previous study[10]. The variation in BNP levels was 12 pg/mL. Using 2-sided type I error of 5% and 90% power with variation of population equal 20, a sample of 30 volunteers was required.

Baseline characteristics were described as number (percentage), median (minimum, maximum), and mean ± standard deviation (SD). The NT-proBNP level was described as mean ± SD at various times, and repeated ANOVA test was employed to test the differences in NT-proBNP levels in the same people with a *p*-value <0.05 taken as significant. The IBM SPSS statistics version 22 was used.

Results

Thirty healthy volunteers were enrolled in the present study. Most (66.7%) were female, and their mean age was 30.30±5.98 years. Mean body weight and height were 58.61±13.00 kilograms and 164.37±8.08 centimeters, respectively, and mean body mass index (BMI) was 21.54±3.57 kg/m^2. Mean serum creatinine was 0.79±0.16 mg/dL while the mean serum AST and serum ALT were 21.80±5.40 IU/L and 22.57±6.51 IU/L, respectively, as shown in Table 1.

NT-proBNP levels were measured five times in the same day. The minimum level was 5 pg/mL and the maximum level was 195 pg/mL. There were 4 subjects who had NT-proBNP level 5 pg/mL and two subjects with maximal NT-proBNP level over 125 pg/mL. The first of these had NT-proBNP levels at 8.00 AM, 12.00 noon, 4.00 PM, 8.00 PM, and 12.00 midnight of 130 pg/mL, 126 pg/mL, 122 pg/mL, 38 pg/mL and 34 pg/mL, respectively, while the other had 57 pg/mL, 19 pg/mL, 195 pg/mL, 192 pg/mL and 104 pg/mL, respectively. The mean NT-proBNP levels at 8.00 AM, 12.00 noon, 4.00 PM, 8.00 PM, and 12.00 midnight were 36.2±29.9 pg/mL, 35.4±30.3 pg/mL, 39.0±40.9 pg/mL, 32.4±36.1 pg/mL and 23.8±20.3 pg/mL, respectively. No significant differences in NT-proBNP levels from the daytime to the nighttime were found (*p*-value = 0.132) as shown in Table 2 and Figure 1.

Table 1. Baseline characteristics data

Factors	Mean ± SD	Median (min, max)
Sex, n (%)		
Female	20 (66.7)	
Male	10 (33.3)	
Age (years)	30.3±5.98	29 (24, 60)
Status, n (%)		
Married	4 (13.3)	
Single	26 (86.7)	
Body weight (kg)	58.61±13.00	56.5 (42, 98)
Height (cm)	164.37±8.08	162.5 (150, 183)
BMI (kg/m^2)	21.54±3.57	20.34 (17.22, 31.11)
Occupation, n (%)		
Doctor	19 (63.3)	
Freelance	2 (6.7)	
Government	6 (20.0)	
Student	1 (3.3)	
Other	2 (6.6)	
Creatinine (mg/dL)	0.79±0.16	0.80 (0.57, 1.1)
AST (IU/L)	21.80±5.40	20 (12, 32)
ALT (IU/L)	22.57±6.51	24 (10, 35)

BMI = body mass index, AST = aspartate aminotransferase, ALT = alanine aminotransferase

Table 2. Results of NT-ProBNP

Time	Mean ± SD (pg/mL)	*p*-value
8.00 AM	36.2±29.9	0.132
12.00 noon	35.4±30.3	
4.00 PM	39.0±40.9	
8.00 PM	32.4±36.1	
12.00 midnight	23.8±20.3	

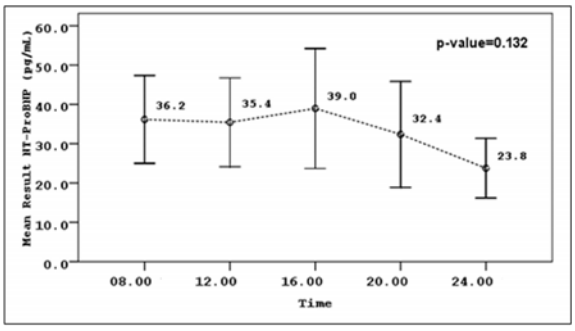

Figure. 1 Results of NT-proBNP at various times of the day.

Discussion

The current clinical practice guidelines recommend that NT-proBNP levels below 125 pg/mL can be used to exclude chronic heart failure[1]. In the present study, however, 2 healthy subjects had the maximum NT-proBNP level of over 125 pg/mL. Both were female and both subjects had NT-proBNP levels at 12.00 midnight is lower than 125 pg/mL; however, elevated NT-proBNP levels are also required the signs and symptoms used to diagnose heart failure. Fradley MG et al[11] showed NT-proBNP values were substantially higher in women than in men at every age, at 42.5 to 106.4 pg/ml in men and 111.0 pg/ml to 215.9 pg/ml in women. Another study[12] revealed NT-proBNP levels in normal healthy women of between 13.6 pg/mL and 126.0 pg/mL with the maximal level over 125 pg/mL, similar to the

results of the present study, and 2 subjects had high levels of NT-proBNP without clinical signs of heart failure. However, NT-proBNP at 12.00 midnight was lower than 125 pg/mL in all healthy volunteers, and this can be taken as an indicator that they were free from chronic heart failure, as in the clinical practice guidelines, with highest specificity.

Bruin S, et al[10] found that there was a difference in NT-proBNP levels at various times of the day. In contrast, other studies[13-18] found that there was no circadian variation of NT-proBNP levels in either healthy or heart-failure patients. The present study did not show any significant difference in circadian variation of NT-proBNP levels, and this is similar to the findings of the aforementioned previous studies[13-18]. These results may be due to the fact that our group was selected from healthy volunteers, who usually have lower levels of NT-proBNP. A more diverse and larger group may have a greater degree of variation and may show a significant difference in NT-proBNP levels during the various times of day.

The present study showed that NT-proBNP levels were highest at 4.00 PM and lowest at 12.00 midnight, but the difference was not significant (p-value = 0.132). In contrast, previous studies[10] found that the lowest level of NT-proBNP at was 8.00 AM and the maximum level at 6.00 PM. This difference may be due to the race and ethnicity of the population groups. The timing of blood sampling was different from the present study in that in the previous study, blood samples were measured only from 8.00 AM to 6.00 PM and no blood samples were taken at 12.00 midnight. In a patient suspected of having heart failure, NT-proBNP levels measured at 4.00 PM may have higher sensitivity than those taken at 12.00 midnight, leading to a false positive diagnosis of heart failure. Further investigations with a larger sample size and including patients with heart failure should be performed.

Limitations

The present study enrolled only healthy volunteers which do not perfectly represent the true general population. The sample size was not large and, as all volunteers were Thai, they were not representative of the worldwide population. NT-proBNP levels after midnight were not measured, and they may not accurately represent the diurnal variation.

Clinical implications

In patients suspected of having heart failure, NT-proBNP levels measured at 12.00 midnight may have a higher specificity than at other times of day, and this may increase false negatives in the test.

Conclusion

The levels of NT-proBNP did not show any significant circadian variation. The levels of NT-proBNP were highest at 4.00 PM which may raise the sensitivity of the test, and lowest at 12.00 midnight, which may increase specificity.

What is already known on this topic?

1) NT-proBNP is used for the diagnosis and prognosis of heart failure.

2) The cut-off point of NT-proBNP for excluding heart failure is 125 pg/mL.

3) Many factors such as age, gender, obesity, concurrent medication and renal function can affect NT-proBNP levels.

4) Circadian variation in NT-proBNP levels is an area of controversy.

What this study adds?

1) No circadian variation of NT-proBNP levels was found in this healthy population.

2) NT-proBNP tended to be highest at 4.00 PM that may increase the sensitivity for diagnosis of heart failure patients.

3) NT-proBNP tended to be lowest at 12.00 midnight, which may increase the specificity for diagnosis of heart failure patients.

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Potential conflict of interest

The authors declare no conflicts of interest.

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