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Diurnal variation of frequency domain T-wave alternans on 24-hour ambulatory electrocardiogram in subjects without heart disease: Significant effect of autonomic nervous activity of the heart

Kenichi Hashimoto¹ | Yuji Kasamaki² | Masayoshi Soma³ | Bonpei Takase¹

¹Department of Intensive Care Medicine, National Defense Medical College, Saitama, Japan

²Department of General Medicine, Kanazawa Medical University Himi Municipal Hospital, Kanazawa, Japan

³Division of General Medicine, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan

Correspondence

Bonpei Takase, Department of Intensive Care Medicine, National Defense Medical College, Saitama, Japan.
Emails: dui15772004@yahoo.co.jp and dui1577@db3.so-net.ne.jp

Abstract

Background: T-wave alternans (TWA) is a risk stratification predictor for sudden cardiac death. However, little is known about the diurnal variation of TWA. Whether TWA are affected by heart rate (HR) or cardiac autonomic nervous activity in the subjects without significant structural heart disease in daily life is not fully understood. Thus, this study was aimed to clarify these issues.

Methods: Frequency domain (FD)-TWA analysis was conducted in 47 subjects without significant structural heart disease using 24-hr ambulatory electrocardiogram (AECG). Measurement of heart rate variability (HRV) was performed in order to evaluate the autonomic activity of the heart. The maximum FD-TWA value in each period was measured four times per day (A, 00:00–6:00 hr; B, 06:00–12:00 hr; C, 12:00–18:00 hr; D, 18:00–24:00 hr). Correlations between FD-TWA and either HR or HRV parameters (LF/HF, LFnu, HFnu, SDNN, CVNN, pNN50) were analyzed in each period (A–D).

Results: There was diurnal variation of FD-TWA (median, inter-quartile range [IQR]: A, 8.2 [6.5, 10.6] μ V; B, 10.1 [8.4, 15.0] μ V; C, 17.6 [12.3, 25.0] μ V; D, 11.9 [9.1, 19.9] μ V; $p < 0.0001$). Maximum FD-TWA had positive correlations with HR and LF/HF (HR, $r = 0.496$, $p < 0.0001$; LF/HF, $r = 0.414$, $p = 0.004$), while FD-TWA had a negative correlation with HFnu ($r = -0.291$, $p = 0.048$). On multiple linear regression analysis, HR had an independent effect on log FD-TWA amplitude ($\beta = 0.461$, $p = 0.001$).

Conclusions: FD-TWA has marked diurnal variation in the daily life of the subjects without significant structural heart disease. This variation could be more strongly affected by HR than the HRV indices.

KEYWORDS

circadian rhythm, heart rate, heart rate variability, Holter monitoring

1 | INTRODUCTION

Microvolt T-wave alternans (TWA), defined as alternating changes of T-wave amplitude or morphology on the electrocardiogram (ECG), has been reported to be a useful predictor of the risk for sudden

cardiac death (SCD) (Smith, Clancy, Valeri, Ruskin, & Cohen, 1988; Rosenbaum et al., 1994; Ikeda et al., 2000) in patients with structural heart disease including ischemic heart disease, dilated cardiomyopathy, and heart failure (Hohnloser, Klingenheden, Bloomfield, Dabbous, & Cohen, 2003; Klingenheden, Zabel,

D'Agostino, Cohen, & Hohnloser, 2000). In the early 2000s, time domain analysis of 24-hr ambulatory electrocardiogram (AECG)-based TWA was introduced. This method is called modified moving averaging of AECG data for 24 hr (Nearing & Verrier, 2002; Verrier et al., 2003). Subsequently, several reports of AECG-based TWA measurements by the time domain method (TD-TWA) showed promising indices for identifying the risk for SCD in post-myocardial infarction and cardiomyopathy patients (Niemenen et al., 2007; Sakaki et al., 2009; Stein, Sanghavi, Sotoodehnia, Siscovick, & Gottdiener, 2010).

Recently, a newly developed AECG system that can provide frequency domain (FD)-TWA analysis has become commercially available (Kawasaki et al., 2015). FD-TWA could be theoretically less affected by noise than TD-TWA (Rashba et al., 2002). Accordingly, FD-TWA measures could decrease false-positive assessments and minimize overestimation of TWA amplitude compared with TD-TWA.

Studies have shown that TWA amplitude is increased by elevations in heart rate (HR) with pacing and exercise (Rosenbaum et al., 1994; Tanno et al., 2004; Turitto et al., 2001), as well as mental stress, while the administration of β -blockers decreases TWA amplitude (Kawasaki et al., 2015; Kop et al., 2004; Selvaraj & Chauhan, 2009). Thus, the strongest independent factor regulating TWA amplitude could be HR in the conventional exercise- or pacing-induced TWA. However, in the TD-TWA measured by the AECG system, a significant cut-off value that is independent of HR has been reported as a predictor of untoward outcome in patients with structural heart disease (Niemenen et al., 2007). In FD-TWA, TWA amplitude is also expected to be independent of HR. In other words, whether or not FD-TWA by AECG is affected by either HR or cardiac autonomic nervous activity expressed as heart rate variability (HRV) has not been fully investigated. In addition, little is known about the diurnal variation of the amplitude of FD-TWA by AECG in subjects without structural heart disease. Any information on what is the significant factor to affect the amplitude of FD-TWA by AECG has not been available till now.

Thus, the purpose of this study was (a) to investigate the existence of diurnal variation of FD-TWA, and (b) to evaluate whether or not HR that is the conventionally significant factor on obtaining TWA, and the autonomic nerve activity of the heart expressed as HRV affect the amplitude of FD-TWA by AECG in daily life in subjects without significant structural heart disease.

2 | METHODS

2.1 | Study population

From 2013 January to 2015 March, consecutive patients who were referred to our outpatient general medicine department to undergo AECG for clinical indications (i.e., screening for

palpitations or chest discomfort) were prospectively enrolled. Before the patients were enrolled in this study, the candidate patients had been clinically evaluated, and they had been followed for at least two months in order to confirm that they had no significant structural heart disease. For the clinical evaluation, the following procedures and examinations were performed: detailed medical history taking, complete physical examination, routine blood tests including biochemistry examination and complete blood cell counts, urinalysis, 12-lead ECG, chest X-ray examination, echocardiography, evaluation with Framingham risk factor (Wilson et al., 1998) and 24-hr AECG. The patients who had never complained of any recurrent symptoms or any recurrent chief complaints without any treatment for a 2-month follow-up period as well as the patients who had not met the following exclusion criteria were enrolled in this study. "The subjects without significant structural heart disease" in this study were defined as patients who did not meet the following exclusion criteria or who did not have any recurrent symptoms for the 2-month follow-up period as mentioned above. The exclusion criteria were as follows: (a) any abnormal findings on past medical history and complete physical examination, (b) any abnormal findings on routine blood tests and urinalysis, (c) any abnormal findings on 12-lead ECG that includes Brugada type ECG, long QT, J wave and early repolarization pattern ECG, (d) any abnormal findings on chest X-ray examination, (e) any abnormal findings on echocardiography, (f) failure to meet the criteria for the "low risk group" defined by the Framingham Heart Study (Wilson et al., 1998), such as normal blood pressure ($<140/90$ mmHg), no dyslipidemia, no history of current smoking habits, no diabetics, and no family history of premature ischemic heart disease (IHD), (g) marked significant ST-T segment change during a 6-min walk, and (h) atrial fibrillation, >500 atrial and/or ventricular extra-systoles per day, and ST segment deviation which met silent myocardial ischemia criteria (Weiner et al., 1991) recorded by 24-hr AECG. By definition, patients taking any cardiovascular medications such as β -blockers, Ca-antagonists, renin-angiotensin-converting enzyme inhibitors, and anti-arrhythmic drugs were excluded in this study.

Among the 138 patients who presented to our clinic with chest symptoms and had AECG recordings available, 91 were excluded based on the exclusion criteria. The remaining 47 subjects were enrolled in this study. As being defined above, our study population was not apparently healthy subjects, but they were considered to be reserve patients for heart disease. In this study, despite our study population being reserve patients for heart disease, we arbitrarily defined our study population as "the subjects without significant structural heart disease" by above mentioned eight exclusion criterion.

Before enrollment of patients for this study, informed consent was obtained from each patient. The study protocol conformed to the ethical guideline of the Declaration of Helsinki and was approved by the ethics committees of Nihon University Hospital and National Defense Medical College Hospital.

2.2 | Echocardiography and 24-hr AECG recording

Echocardiography was performed using the Acuson SC 2000 system (Siemens Co., Ltd., Erlangen, Germany), and the data were recorded in DICOM format. The left ventricular ejection fraction (LVEF) was calculated using Simpson's formula during sinus beats. Routine AECG parameters (total heartbeats, maximum HR, minimum HR, mean HR, the numbers of atrial premature contractions [APCs] and ventricular premature contractions [VPCs], and ST-T segment changes) were recorded by an FM180S (Fukuda Denshi Co., Ltd., Tokyo, Japan) and analyzed by AECG system SCM 8,000 (Fukuda Denshi Co., Ltd.). In this study, orthogonal X, Y, and Z bipolar leads were utilized for AECG recording.

2.3 | Analysis of FD-TWA

TWA analysis was also performed by the FM180S (Fukuda Denshi Co., Ltd.) and its dedicated analyzer, SCM 8,000 (Fukuda Denshi Co., Ltd.). Silver-silver chloride electrodes (Magunerode TE-18, Fukuda Denshi Co., Ltd.) were used, which were suitable for high-resolution ECG recording. The technique of FD-TWA in this study was based on the real-time spectral method of TWA that has been published by several investigators (Rosenbaum et al., 1994; Smith et al., 1988). In the present study, the spectral method of the TWA measurement was modified for the AECG recordings (Kawasaki et al., 2015). The T-wave data were filtered through a low-pass band (300 Hz) filter, and digital sampling was conducted at 1,000 Hz. By using the QRS complex as the trigger, the corresponding five points of T-wave amplitude were calculated for spectral analysis. Then, 128 consecutive beats of T waves were averaged and superimposed on each of the five points on one T wave. The fluctuation of T-wave amplitude was computed using fast Fourier transformation. A spectral power of 0.5 cycles/beats was obtained for FD-TWA. The alternans power (μV^2) was defined as the difference between the power at the alternans frequency (0.5 cycles/beat) and the power at the noise frequency point. The alternans voltage (μV) was equal to the square root of alternans power. The alternans ratio (R_{alt}), calculated as the alternans power divided by the standard deviation of the noise in the reference frequency band, was obtained. Significant FD-TWA was defined as (R_{alt}) >3.0 in more than one of the orthogonal bipolar X, Y, and Z leads lasting for more than 30 s. In addition, a significant TWA measurement was defined as <10% invalid beats (i.e., APCs and VPCs, etc.) and a noise level <20 μV over the 128 beats for calculation of the TWA. The FD-TWA data detected at HRs less than 120 beats per minute were used. The results of FD-TWA were over-read by two independent investigators, and disagreements were resolved by consensus. To investigate diurnal variation, the maximum FD-TWA value in each period was measured four times per day (00:00–06:00, 06:00–12:00, 12:00–18:00, and 18:00–24:00 hr).

2.4 | Analysis of HRV

For evaluation of cardiac autonomic tone activity, HRV analyses were also performed with a dedicated analyzer (SCM 8000). The RR

interval was used to evaluate autonomic activity by the corrected maximum entropy method using Akaike's algorithm, as reported previously (Mizumaki et al., 2004). HRV for the frequency domain analysis was analyzed for every five-minute interval. Power spectra were measured by the area within the following frequency bands: total power (TP), 0.0001–0.5 Hz; very low frequency range (VLF), 0–0.04 Hz; low frequency range (LF), 0.04–0.15 Hz; and high frequency range (HF), 0.15–0.40 Hz. The LF, HF, and LF/HF ratio were determined every five minutes. The LF normalized unit (LFnu) was calculated as $LF/TP \times 100$, and the HF normalized unit (HFnu) was calculated as $HF/TP \times 100$, according to the previously published report (Task Force of the European Society of Cardiology & the North American Society of Pacing & Electrophysiology, 1996). For the time domain analysis, the parameters of the standard deviation of the mean normal RR intervals for 5-min segments (SDNN, ms), the estimate of the coefficient of variation of the RR interval (CVNN, %), and the percentage of the differences between adjacent normal RR intervals >50 ms computed over the 24-hr AECG (pNN50, %) were also measured every five minutes.

2.5 | Measurement of correlations between FD-TWA and either HR or HRV

Maximum FD-TWAs in each time period (00:00–6:00, 06:00–12:00, 12:00–18:00, and 18:00–24:00 hr) were measured with manual editing (four points per day) to assess the correlations between FD-TWA and HR, and the correlations between FD-TWA and HRV indices (SDNN, pNN50, CV, LF/HF, LFnu, HFnu) were also evaluated. The mean HR and HRV indices were also calculated for five minutes including the same time when the maximum FD-TWA was observed.

Moreover, the maximum FD-TWA over 24 hr (one point per day) was measured to assess the correlations between FD-TWA and the mean HR where maximum FD-TWA was observed, as well as the correlations between FD-TWA and the HRV indices. The mean HR and HRV indices were also calculated for five minutes including the same segments of the maximum FD-TWA observed over 24 hr.

2.6 | Statistical analysis

Continuous values are appropriately expressed as means $\pm$ standard deviation and/or medians with 25th and 75th percentiles. Friedman's analysis of ANOVA on rank was used for the comparisons of FD-TWA, the HRV indices (SDNN, CVNN, pNN50, LF/HF, LFnu, HFnu), and HR for each time interval (00:00–6:00, 06:00–12:00, 12:00–18:00, and 18:00–24:00 hr). The frequency of FD-TWA in each period was analyzed by chi-squared tests. Spearman's correlation coefficient analysis was used to assess the correlations between FD-TWA and HR, as well as the correlations between FD-TWA and the HRV indices. Before performing multiple regression analysis, FD-TWA and the HRV indices (SDNN, CVNN, pNN50, LF/HF, LFnu and HFnu) were transformed to natural logarithms because these parameters showed lognormal distributions. All parameters with $p < 0.1$ on univariate analysis were included in the multiple

TABLE 1 (A) Maximum FD-TWA amplitude in each time period and HR when the maximum FD-TWA was observed. (B) HRV (time domain analysis) measures when the maximum FD-TWA was observed in each time period. (C) HRV (frequency domain analysis) measures when the maximum FD-TWA was observed in each time period

Sex	00:00–06:00	06:00–12:00	12:00–18:00	18:00–24:00	p
A					
FD-TWA (μ V)					
Total	8.2 (6.5, 10.6)	10.1 (8.4, 15.0)	17.6 (12.3, 25.0)	11.9 (9.1, 19.9)	<0.0001
Male	8.3 (5.5, 11.3)	9.3 (5.1, 14.3)	17.1 (12.4, 22.0)	12.6 (8.1, 20.9)	<0.0001
Female	8.5 (6.7, 10.4)	10.2 (8.9, 15.4)	18.6 (12.1, 28.4)	11.7 (9.7, 19.8)	<0.0001
HR (bpm)					
Total	64.9 $\pm$ 12.6	80.8 $\pm$ 18.8	91.3 $\pm$ 15.1	76.5 $\pm$ 14.6	<0.0001
Male	65.7 $\pm$ 15.3	74.8 $\pm$ 18.0	88.2 $\pm$ 16.2	75.8 $\pm$ 12.9	<0.0001
Female	64.3 $\pm$ 15.3	84.9 $\pm$ 18.5	93.4 $\pm$ 14.2	77.0 $\pm$ 15.9	<0.0001
B					
SDNN (ms)					
Total	47.50 (32.50, 73.25)	42.50 (31.75, 67.25)	39.50 (23.50, 53.25)	50.88 (36.00, 60.25)	0.039
Male	56.00 (36.00, 122.00)	50.00 (32.00, 74.00)	41.00 (29.00, 56.00)	52.00 (48.00, 61.00)	0.049
Female	42.00 (27.00, 60.50)	37.50 (27.25, 66.25)	35.00 (20.5, 43.50)	40.50 (32.70, 57.00)	0.138
CVNN (%)					
Total	5.88 (3.68, 7.79)	6.89 (5.01, 7.80)	6.29 (4.09, 7.74)	6.28 (4.60, 8.32)	0.183
Male	7.14 (5.25, 10.27)	7.12 (5.15, 8.19)	6.12 (5.42, 8.90)	6.98 (6.00, 10.24)	0.528
Female	4.26 (2.86, 6.42)	5.32 (4.10, 8.05)	5.85 (3.61, 7.28)	6.04 (4.82, 7.05)	0.182
pNN50 (%)					
Total	5.39 (1.47, 16.87)	1.30 (0.00, 12.44)	0.37 (0.00, 2.24)	3.55 (0.78, 14.12)	<0.0001
Male	5.65 (3.04, 36.36)	3.46 (0.84, 33.80)	1.28 (0.24, 8.39)	6.09 (3.16, 25.70)	0.001
Female	5.09 (0.47, 11.78)	0.37 (0.00, 4.44)	0.00 (0.00, 0.96)	1.68 (0.00, 4.70)	0.013
C					
LF/HF					
Total	1.83 (0.90, 2.87)	2.69 (1.72, 5.40)	3.90 (2.55, 6.17)	3.16 (2.00, 4.71)	<0.0001
Male	1.50 (0.93, 2.75)	2.30 (1.20, 5.77)	3.47 (2.31, 6.33)	2.88 (2.00, 3.94)	0.028
Female	1.92 (0.90, 2.80)	3.49 (2.33, 5.58)	4.04 (3.03, 6.44)	3.89 (2.32, 5.90)	0.034
LFnu					
Total	26.48 (15.36, 44.21)	27.00 (14.50, 46.34)	25.10 (16.00, 32.35)	29.02 (18.20, 45.49)	0.280
Male	21.57 (14.23, 38.06)	29.57 (20.45, 39.38)	26.28 (16.89, 31.82)	23.43 (16.55, 37.42)	0.877
Female	30.22 (21.58, 48.37)	29.92 (11.70, 47.66)	23.85 (15.24, 41.93)	36.13 (19.89, 49.92)	0.086
HFnu					
Total	18.70 (16.72, 0.27)	9.49 (0.05, 0.20)	6.05 (3.15, 9.48)	9.50 (4.49, 15.58)	<0.0001
Male	13.10 (6.32, 23.55)	12.69 (6.65, 20.74)	7.78 (3.44, 11.04)	10.56 (5.91, 13.77)	0.012
Female	20.24 (12.98, 28.31)	6.14 (3.6, 13.52)	4.50 (3.1, 9.09)	8.60 (4.02, 16.05)	0.001

Note. A: Values of FD-TWA are presented as medians (1st, 3rd quartiles). Values of HR are presented as means $\pm$ standard deviation. B and C: Values are presented as medians (1st, 3rd quartiles).

CVNN (%): the estimation of coefficient of variation of R-R intervals; FD-TWA: Frequency domain T-wave alternans; HFnu: high frequency range normalized unit; HR: Heart rate; HRV: heart rate variability; LF/HF: low frequency range/high frequency range; LFnu: low frequency range normalized unit; pNN50 (%): percentage of differences between adjacent normal RR intervals that are >50 ms computed over 24 hr; SDNN: standard deviation of the mean normal RR intervals.

regression model. However, upon theoretical consideration of the important factors that are highly related to diurnal variation of TWA, we also selected sex and age as the explanatory variables. Then, multiple linear regression analysis with stepwise selection was

also performed to determine the intensities of FD-TWA among the HR and HRV indices. These univariate and multiple analyses were performed both in each time period (00:00–06:00, 06:00–12:00, 12:00–18:00 and 18:00–24:00 hr) and throughout the 24 hr for the

maximum FD-TWA. SPSS version 22 (IBM Corp, Armonk, NY) was used for statistical analysis. In all analyses, $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | Clinical characteristics in study population, the results of routine clinical evaluation and echocardiographic findings

Forty-seven subjects (20 men and 27 women; mean age 44.3 ± 18.1 years; range 21–83 years) were enrolled in this study. The age distribution was as follows: 23 subjects were young (<45 years old); 20 were middle aged (≥ 45 but <65 years old); and four were of advanced age (≥ 65 years old). The body mass index (BMI) of the 47 subjects was 22.4 ± 3.9 (range 17–31 kg/m²): with 22 subjects having a BMI <25 kg/m²; 23 having a BMI ≥ 25 but <30 kg/m²; and two having a BMI ≥ 30 kg/m². As being mentioned in the method section, complete physical examination, routine blood tests, urinalysis, 12-lead ECG, and chest X-ray examination showed no abnormal findings by definition. The echocardiographic main findings were as follows: the mean LVEF calculated by Simpson's formula was $70.2\% \pm 7.6\%$, the mean left atrial diameter was 30.1 ± 5.6 mm, left ventricular end-systolic diameter was 45.6 ± 3.6 mm, left ventricular end-systolic diameter was 27.5 ± 4.0 mm, and the other parameters were also within normal ranges.

3.2 | Routine analysis of 24-hr AECG

The analysis of AECG recorded in the 47 present study subjects showed following findings. That is, total heartbeats were $104,129.7 \pm 23,005.0$ beats per day. Maximum HR was 131.2 ± 25.4 bpm, minimum HR was 51.2 ± 6.4 bpm, and average HR was 76.9 ± 8.4 bpm, respectively. The number of APCs was 86.9 ± 55.3 beats per day, and that of VPCs was 58.5 ± 32.4 beats per day.

3.3 | Outcomes and diurnal variation of FD-TWA, HR, and HRV indices

Table 1 shows FD-TWA, HRV, and HR in each time interval. Diurnal variations of FD-TWA and HR were observed in both men and women in Table 1A. The largest FD-TWA amplitude of all subjects was observed during 12:00–18:00 hr. The minimum value was obtained during 00:00–06:00 hr ($p < 0.0001$) as shown in Table 1A. And Figure 1 that showed individual values TWA in each subject. The HR when the maximum FD-TWA was observed also showed diurnal variation as shown in Table 1. The HR and HRV indices (time domain analysis, SDNN, pNN50; frequency domain analysis, LF/HF, LFnu, and HFnu) also showed significant diurnal variations as shown in Table 1B,C. However, CVNN and LFnu did not show diurnal variations. Therefore, the indices of CVNN and LFnu were excluded from the further analysis in this diurnal variation study.

FIGURE 1 Diurnal variation of FD-TWA in each subject. Median values of frequency domain T-wave alternans (FD-TWA) amplitude were largest during 12:00–18:00 and smallest during 00:00–06:00. FD-TWA, frequency domain T-wave alternans

3.4 | Correlations between FD-TWA and either HR or HRV indices in each time period

The relationships between FD-TWA amplitude and HR and between FD-TWA magnitude and the HRV indices (SDNN, pNN50, LF/HF, HFnu) were evaluated and showed diurnal variations as shown in Table 2. On univariate analysis, there were positive correlations between TWA and HR during three time periods. Positive correlations were observed between FD-TWA and LF/HF during two time periods, whereas there was a negative correlation between FD-TWA and HFnu during three time periods. In addition, there were no correlations between FD-TWA and CVNN or pNN50 (Table 2). On multiple linear regression analysis with stepwise selection including sex and age as the explanatory variables, HR independently affected FD-TWA amplitude during all time periods as shown in Table 3.

3.5 | Correlations between maximum FD-TWA over 24 hr and either HR or HRV

The frequencies of maximum FD-TWA amplitudes observed in each time period as illustrated in Figure 2. Maximum FD-TWA amplitudes were observed the most frequently during 12:00–18:00 hr, followed by during 18:00–24:00 and during 06:00–12:00 hr. The lowest amplitude was observed during 00:00–6:00 hr. The relationships between maximum FD-TWA amplitude throughout the day and HR over 24 hr and relationships between maximum FD-TWA amplitude and the HRV indices (SDNN, pNN50, LF/HF, HFnu) were evaluated. There was a significant positive correlation between TWA and HR as shown in Figure 3 and Table 4. The significant positive correlations were observed between FD-TWA and LF/HF, while the significant negative correlation between FD-TWA and HFnu was observed in Table 4. On multiple linear regression analysis including all variables with p values <0.1 on univariate analysis, and sex and age, HR

	00:00–06:00 (n = 47)	06:00–12:00 (n = 47)	12:00–18:00 (n = 47)	18:00–24:00 (n = 47)
FD-TWA				
<i>r</i>	1.000	1.000	1.000	1.000
<i>p</i>	—	—	—	—
Age				
<i>r</i>	−0.137	−0.204	0.79	0.104
<i>p</i>	0.369	0.174	0.625	0.496
Sex				
<i>r</i>	0.086	−0.40	−0.112	−0.226
<i>p</i>	0.575	0.794	0.486	0.145
HR				
<i>r</i>	0.249	0.599**	0.431**	0.563**
<i>p</i>	0.098	<0.001	0.004	<0.001
SDNN				
<i>r</i>	0.017	−0.120	−0.048	−0.008
<i>p</i>	0.912	0.442	0.764	0.957
pNN50				
<i>r</i>	0.004	−0.231	−0.105	−0.262
<i>p</i>	0.979	0.142	0.512	0.083
LF/HF				
<i>r</i>	0.040	0.339*	0.172	0.478**
<i>p</i>	0.793	0.024	0.283	0.001
HFnu				
<i>r</i>	0.092	−0.456**	−0.309*	−0.439**
<i>p</i>	0.548	0.002	0.049	0.003

Note. FD-TWA: frequency domain T-wave alternans; HFnu: high frequency range normalized unit; HR: heart rate; HRV: heart rate variability; LF/HF: low frequency range/high frequency range; pNN50 (%): percentage of differences between adjacent normal RR intervals that are >50 ms computed over 24 hr; SDNN: standard deviation of the mean normal RR intervals.

p* < 0.05. *p* < 0.01.

independently affected the maximum FD-TWA amplitude over 24 hr as shown in Table 5.

4 | DISCUSSION

In this study, diurnal variation of the amplitude of FD-TWA by AECG was seen in “the subjects without significant structural heart disease” during daily life for 24 hr. Furthermore, FD-TWA amplitude was more strongly affected by HR rather than the other HRV indices.

4.1 | Diurnal variation of the amplitude of FD-TWA by AECG

Diurnal variation of FD-TWA by AECG was seen in “the subjects without significant structural heart disease” as seen in Table 1A, Figures 1, and 2. In detail, median FD-TWA amplitude and the frequency of maximum FD-TWA were highest in the daytime (12:00–18:00), while they were lowest at midnight (00:00–6:00). The amplitude of

TABLE 2 Correlations between FD-TWA and HR/HRV indices in each time period (univariate analysis)

FD-TWA by AECG is more strongly affected by HR than by the other HRV indices. This finding is well comparable to the correlation between HR and microvolt TWA with other spectral methods (Kavesh, Shorofsky, Sarang, & Gold, 1998).

The existence diurnal variation of FD-TWA by AECG in this study could be reasonably speculated by the following previous reports on the clinical diurnal variation of SCD onset even if the study population is different. Diurnal variation of out-of-hospital SCD in ventricular fibrillation was reported by Arntz et al. (1993) and Chugh et al. (2008). According to their investigations, two peaks of out-of-hospital SCD incidence were observed. The first peak of SCD was observed in the morning starting at 6 a.m., and the second peak was observed during the afternoon. In the present result, FD-TWA amplitude showed afternoon dominance. The second peak time of SCD incidence was consistent with the peak time when the maximum amplitude of FD-TWA was observed over 24 hr in our study. It has been reported that FD-TWA was useful to detect those at high risk of malignant ventricular tachyarrhythmias leading to SCD (Kawasaki et al., 2015).

TABLE 3 Multiple regression analysis of HR and HRV indices according to the intensity of FD-TWA amplitude in each time period

	$R^2 = 0.203$		$R^2 = 0.106$		$R^2 = 0.331$		$R^2 = 0.308$	
	β	p	β	p	β	p	β	p
log FD-TWA	—	—	—	—	—	—	—	—
Age	-0.247	0.134			-0.060	0.685		
Sex	0.098	0.549			-0.087	0.552		
HR	0.296	0.155	0.325	0.038 ^a	0.504	0.016	0.555	<0.001 ^a
log pNN50	0.051	0.794			-0.074	0.736		
log LF/HF	0.352	0.208			-0.178	0.445		
log HFnu	0.344	0.208			-0.183	0.404		
12:00–18:00								
	$R^2 = 0.439$		$R^2 = 0.241$		$R^2 = 0.416$		$R^2 = 0.271$	
	β	p	β	p	β	p	β	p
log FD-TWA	—	—	—	—	—	—	—	—
Age	-0.540	0.709			0.027	0.860		
Sex	-0.780	0.564			-0.261	0.088		
HR	0.628	<0.001	0.491	0.020 ^a	0.523	0.006	0.521	0.001 ^a
log pNN50	0.417	0.130			0.264	0.139		
log LF/HF	-0.090	0.960			0.201	0.316		
log HFnu	-0.309	0.069			-0.128	0.463		

Note. FD-TWA: frequency domain T-wave alternans; HR: heart rate; HRV: heart rate variability; log HFnu: natural logarithmic transformation of the high frequency range normalized unit; Log LF/HF: natural logarithmic transformation of the low frequency range normalized unit/high frequency range normalized unit; log pNN50 (%): natural logarithmic transformation of percentage of differences between adjacent normal RR intervals that are >50 ms computed over 24 hr.

^aVariables by multiple linear regression with stepwise selection.

4.2 | Impacts of HR and autonomic activity on FD-TWA

The amplitude of FD-TWA by AECG is more strongly affected by HR than by the other HRV indices. As seen in Table 1A, the HR also showed diurnal variation. The pattern of diurnal variation in HR was similar to that in FD-TWA. As shown in Tables 2 and 3, FD-TWA amplitude was more strongly affected by HR than the other HRV indices in all time periods. Furthermore, HR was the strongest factor affecting maximum FD-TWA over 24 hr as shown in Tables 4 and 5. Since HR was only significant factor according to the intensity of FD-TWA in multiple regression models with stepwise selection through all time periods (Table 3) and over 24 hr (Table 5), HR might be the most important factor regulating the diurnal variation of FD-TWA by AECG.

Our study results suggest that the amplitude of FD-TWA by AECG in daily life in “the subjects without significant structural heart disease,” HR is more important factor to determine the amplitude of FD-TWA by AECG than HRV indices reflecting cardiac autonomic nerve activity. It has been reported that either HR (Tanno et al., 2004; Turitto et al., 2001) or cardiac autonomic nerve activity could affect TWA amplitude (Klingenheben et al., 2000; Kop et al., 2004; Rashba et al., 2002). It is well known that TWA amplitude is augmented by HR rising in conventional TWA measure. However, it has been reported that accentuated

FIGURE 2 The frequency of maximum FD-TWA over 24 hr (1 point/subject/24 hr). Maximum frequency domain T-wave alternans (FD-TWA) amplitude were most frequently observed during 12:00–18:00 in 53.2% (25/47), followed by 18:00–24:00 in 25.5% (12/47) and 06:00–12:00 in 17.0% (7/47), and the lowest was observed during 00:00–06:00 in 4.3% (2/47). FD-TWA: frequency domain T-wave alternans

sympathetic nerve activity can enlarge the TWA amplitude without HR rising in the TD-TWA by 24-hr AECG (Kop et al., 2004). In this report, the practice of mental arithmetic stress such as

complicated calculation or anger emotion significantly increased TD-TWA amplitude even when TD-TWA amplitude was adjusted by HR with pacing in the IHD patients with implantable cardioverter defibrillator (ICD). These results have suggested that the increment of TWA and electrical instability were caused by another independent pathway of central autonomic regulation related to mental stress (Kop et al., 2004). As far as we have searched the previous literatures, this might be the first report to have evaluated simultaneously the correlations between FD-TWA by AECG and either HR or the HRV indices in daily life of the relatively normal patients similar to “the subjects without significant structural heart disease” in the present study. Therefore, our study results might provide the important information regarding pathophysiologic mechanisms of repolarization abnormality expressed as abnormal amplitude of FD-TWA by AECG.

4.3 | FD-TWA by AECG versus conventional standard spectral TWA measurements

The values obtained using FD-TWA by AECG reasonably differ from those of conventional spectral TWA. First, the equipment and devices for measuring FD-TWA are quite different from those used for conventional spectral TWA measurements. The only identical

FIGURE 3 The correlation between maximum FD-TWA amplitude and heart rate. There was a positive correlation between maximum FD-TWA and heart rate ($r = 0.496$, $p < 0.0001$). FD-TWA: frequency domain T-wave alternans

	FD-TWA	Age	Sex	HR	SDNN	pNN50	LF/HF	HFnu
r	1	-0.15	0.110	0.496**	-0.226	-0.162	0.414**	-0.291*
p	—	0.923	0.461	<0.0001	0.126	0.282	0.004	0.048

Note. FD-TWA: frequency domain T-wave alternans; HFnu: high frequency range normalized unit; HR: heart rate; HRV: heart rate variability; LF/HF: low frequency range normalized unit/high frequency range normalized unit; pNN50 (%): percentage of differences between adjacent normal RR intervals that are >50 ms computed over 24 hr; SDNN: standard deviation of the mean normal RR intervals.

* $p < 0.05$. ** $p < 0.01$.

TABLE 5 Multiple regression analysis of HR and HRV indices according to the intensity of the maximum FD-TWA over 24 hr

	$R^2 = 0.325$		$R^2 = 0.224$	
	β	p	β	p
log FD-TWA	—	—	—	—
Age	0.045	0.749		
Sex	0.018	0.895		
HR	0.386	0.007	0.474	0.001 ^a
log LF/HF	0.200	0.250		
log HFnu	-0.176	0.297		

Note. FD-TWA: frequency domain T-wave alternans; log FD-TWA: natural logarithmic transformation of FD-TWA. HR: heart rate; HRV: heart rate variability; log LF/HF: natural logarithmic transformation of the low frequency range/high frequency range; log HFnu: natural logarithmic transformation of the high frequency range normalized unit.

^aVariables by multiple linear regression with stepwise selection.

element between the two is the algorithm of the spectral methodology. Specifically, the electrical lead that is normally used in high-resolution ECG recording and the other instruments for FD-TWA measurements differ extensively from those used for conventional spectral TWA measurements. In addition, the length of the measurement period between FD-TWA by AECG and conventional spectral TWA is very different. Therefore, it is difficult to apply the positive criteria of conventional spectral TWA to that in FD-TWA by AECG. If the results of the present study are considered, the maximum FD-TWA by AECG ranges from 8.2 to 17.6 μV . This range of the amplitude of FD-TWA by AECG is much larger than that in the positive voltage criteria of conventional spectral TWA, that is >1.9 μV . Direct comparison between conventional spectral TWA and FD-TWA by AECG had previously been conducted in patients with structural heart disease, including myocardial infarction and cardiomyopathy (Hoshida et al., 2011). In this previous study, conventional spectral TWA and FD-TWA by AECG were simultaneously measured in the same patients. The maximum value of the conventional spectral method was $6.8 \pm 1.7 \mu\text{V}$, whereas the maximum value of FD-TWA by AECG was $20.2 \pm 8.8 \mu\text{V}$. The value of FD-TWA was almost three times larger than the value of conventional spectral TWA, even if a relatively good correlation between the value of FD-TWA by AECG and that of conventional spectral TWA was observed (correlation coefficient = 0.57). It is speculated that the different values between FD-TWA by AECG and conventional spectral TWA are caused by a combination of the multiple factors mentioned above.

TABLE 4 Correlations between maximum FD-TWA over 24 hr and HR/HRV indices (univariate analysis)

4.4 | Clinical implications

The present data suggest that FD-TWA by AECG should be analyzed and read mainly during the daytime, especially between 12:00 and 18:00 because the maximum FD-TWA value was seen during this time period, as shown in Figure 2. Therefore, according to the results of the present study, the likelihood of missing the maximum FD-TWA could be greatly decreased by reading FD-TWA mainly during the daytime. Since cardiologists and technicians should routinely analyze and read the AECG data as part of their daily work, focusing on just the data collected during the daytime will save time. Therefore, the present results showing diurnal variation of FD-TWA by AECG could be useful information in clinical practice even if our study population consisted of “the subjects without significant structural heart disease.”

4.5 | Limitations

There are several limitations in this study. First, the study population was small and did not contain any healthy volunteers. In addition, this study is a single center, cross-sectional observation. Therefore, we could not give normal limits or reference values of FD-TWA by AECG or pathological cut-off values of FD-TWA by AECG in patients with structural heart disease. Additional studies of FD-TWA by AECG in a larger population of healthy volunteers with a wider range of ages should be conducted in the future. Second, FD-TWA by AECG could not be analyzed when the noise level of FD-TWA was high (above 20 μ V) during the daytime. Therefore, FD-TWA could not be fully evaluated for 24 hr. There might be some possibility that the results for maximum FD-TWA amplitude were underestimated. However, noise effects are a common limitation of studies using AECG. Further technological improvement should be expected to reduce noise in the future. Third, the present study could not thoroughly exclude the possibility of some degree of latent IHD or other heart diseases, such as inherited heart disease (e.g., Brugada syndrome), long QT syndrome, idiopathic ventricular fibrillation, and early repolarization syndrome in the study population. For this reason, we referred to our patient population as subjects without significant structural heart disease, and not as normal healthy subjects. However, we excluded patients in the mid- to high-risk group based on the Framingham risk factor as well as patients exhibiting any ECG abnormalities, so the likelihood of IHD or other arrhythmic disorders in our study population is considered low. Lastly, another important problem to consider regarding the possible clinical significance of TWA is that it is not necessarily the same in patients with and without heart disease. TWA in patients with heart disease is established as a predictor of sudden cardiac death; however, whether this is true for patients without heart disease is unknown. For example, in healthy people, daily fluctuation of TWA might be a physiological advantage, rather than a risk for sudden death. As an already established concept on HRV (Task Force of the European Society of Cardiology & the North American Society of Pacing &

Electrophysiology, 1996), the decreased physiological fluctuation of HR is closely associated with an adverse outcome of cardiovascular diseases. Thus, the role of diurnal variation of TWA on a sudden cardiac death predictor is not determined in this study. The further study is necessary on this problem.

5 | CONCLUSIONS

There is significant diurnal variation of FD-TWA by AECG, which is different from conventional spectral TWA, in “the subjects without significant structural heart disease.” FD-TWA amplitude by AECG is most affected by HR in this population whereas the correlation of FD-TWA with HRV indices that reflect cardiac autonomic activity is relatively weak.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Kenichi Hashimoto <http://orcid.org/0000-0001-6838-1215>

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