Relationship Between Heart Rate Variability and Angiotensinogen Gene Polymorphism in Diabetic and control individuals

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Abstract— **Heart Rate Variability (HRV) is extensively used to investigate general Autonomic Nervous System (ANS) function and is affected by many factors including age, gender, pathology such as diabetes and genetic polymorphisms. One of these genetic polymorphisms is the Angiotensin Converting Enzyme (ACE) polymorphism corresponding to insertion (I) or deletion (D) of a 287-base pair sequence of DNA in intron 16 of the ACE gene (rs4340). Some studies have addressed the relationship between HRV and D/D, I/D and I/I ACE polymorphism while others combined I/D and I/I ACE groups. In this study HRV is determined for diabetic and control individuals with different ACE polymorphism considering either separate or combined I/D and I/I genotypes. Linear time domain parameters, entropy, low frequency and total power of HRV were found to be significantly different between diabetic and control individuals with combined I/I and I/D ACE polymorphism, while only entropy was different for diabetic and control subjects with D/D ACE genotype. Separate analysis of I/I and I/D genotypes was preferred for a thorough investigation of HRV and ACE polymorphism, as the combined analysis masked some differences in HRV parameters such as Poincaré plot between ACE polymorphisms and diabetes status. Furthermore, a separate analysis demonstrated that most of the significant differences for HRV were between the diabetic group with I/I genotype and I/D and D/D groups.**

I. INTRODUCTION

Hear Rate Variability (HRV) is used as an index of the Autonomic Nervous System (ANS) general function and its role in controlling heart rate [1]. HRV is characterized by linear or nonlinear time domain or frequency domain features.

Time domain features of HRV include mean and standard deviation of normal inter-beat (NN) intervals (SDNN) and the root-mean square of NN intervals (RMSSD) [2], a reduction of which was reported to lead to a higher mortality risk in conditions associated with an autonomic imbalance [3], [4]. Tone- Entropy (T-E) analysis is also a time domain measure, which provides information both on sympatho-vagal balance and total activity of the heart rate over the recording period [5]. Poincaré plot provides a more detailed HRV analysis by demonstrating the patterns of heart rate dynamics in the Cartesian plane and representing its long and short-term variability [6].

The use of frequency domain measures including the high frequency component (HF, above 0.15 Hz), low (LF, 0.04 - 0.15 Hz) and very low (VLF, 0.003-0.04 Hz) frequency component, and their ratio (LF/HF) improved the sensitivity of identifying pathological ANS imbalance [7], [8].

HRV can is affected by various factors such as age, gender and some diseases including diabetes [9], [10]. For example HRV parameters including SDNN, PNN50% and RMSSD in diabetic patients are significantly different from control [10]. Genetic factors also affect HRV [11], [12] which include the Angiotensin Converting Enzyme (ACE) polymorphism corresponding to insertion (I) or deletion (D) of a 287-base pair sequence of DNA in intron 16 of the ACE gene (rs4340) [13] and its influence on HRV has been addressed in a number of studies [14]–[17]. These studies analyzed HRV for either three groups: D/D, I/D, I/I genotypes or two groups: D/D and combined I/D and I/I genotypes [14], [16], [18].

In the current paper we discuss the influence of ACE genotype on HRV depending on whether the subjects were divided into three or two ACE genotype groups, in conjunction with the effect of diabetes.

The paper is organized as follows. Section II describes the methods, including details about subjects, genotyping, grouping, preprocessing and HRV analysis. Section III presents the results of HRV analysis and comparison of groups with different ACE genotypes and diabetic status. Section IV is devoted to the discussions on findings and comparisons. Conclusion can be found in Sections V.

II. METHODS

A. Subjects

ECG recordings, ACE genotypes, diabetes details and demographic information were collected from 231 participants at the Charles Sturt University Diabetes Complications Screening Initiative (DiScRi). Written, informed consent approved by the respective local clinical research ethics committees, was provided prior to participating in this study. Five cases with type I diabetes or prediabetes were excluded and only Type II diabetes (T2D) and normal subjects were studied. Equal tachogram length of 500 RR intervals was

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TABLE I
DEMOGRAPHIC DETAILS OF DIFFERENT GROUPS.

Groups	Total No.	Gender No. (%)	Age (years)
D/D, control	38	M:16(42%)- F:22(58%)	67 ± 10
D/D, T2D	16	M:6(38%)- F:10(62%)	66 ± 10
I/D, control	91	M:39(43%)- F:52(57%)	66 ± 11
I/D, T2D	36	M:19(53%)- F:17(47%)	69 ± 8
I/I, control	31	M:11(35%)- F:20(65%)	65 ± 10
I/I, T2D	13	M:5(38%)- F:8(62%)	70 ± 7
I/D+I/I, control	123	M:50(41%)- F:73(59%)	66 ± 11
I/D+I/I, T2D	49	M:24(49%)- F:25(51%)	70 ± 7

Age is shown as mean ± SD.

analyzed for each subject and five cases were excluded because of insufficient tachogram length.

B. Genotyping

The QIAamp DA blood mini kit (from Qiagen) was used to extract genomic DNA from frozen blood samples, according to the manufacturer's instructions. Seventy five microliter of elution buffer was used instead of 200 microliter as the only modification to the protocol in order to obtain a more concentrated solution of DNA. The extracted DNA was then genotyped using the triple primer method [19] and electrophoresis using a 6% polyacrylamide gel.

C. Groups

Subjects were divided in two ways; six groups with ACE genotypes of D/D, I/D and I/I, and all three groups further divided in to those with T2D and control; or four groups in which I/I and I/D were combined and again subdivided into T2D and control. The number of subjects in each group is summarized in table I.

D. ECG recording and preprocessing

ECGs were recorded by trained staff on Powerlab using the sampling rate of 400 Hz and a notch filter at 50 Hz (ADInstruments, Sydney) from a lead II configuration. Patients rested for 5 minutes before measurement and data were collected between 9-11 am for a period of 10 or 20 minutes from 231 individuals while resting in a supine position. The ECGs were edited using the MLS310 HRV module as part of the Powerlab Chart recording software (ADInstruments, Sydney). The RR intervals were determined as the time difference between two successive R peaks in seconds using the method proposed by Pan and Tomkin [20].
Automated adaptive preprocessing was then employed to detect and replace the false beats. The process was performed in three steps described in [21]. First the obvious errors which were shorter than 200 ms long RR intervals were deleted. Then an adaptive percent filter was applied by passing the sequence through a binomial filter and obtaining the adaptive mean and standard deviation (SD) values for each RR interval. Finally the adaptive controlling filter was applied to the resulting sequence and the mean and SD were calculated for each RR interval. Step 2 and 3 identified further ectopic beats, which were then replaced by the respective value of the filtered sequence [21].

E. HRV analysis

1) Conventional time and frequency domain methods: Several time domain HRV features such as: mean and SD of RR intervals (SDNN) and the square root of the mean squared difference of the successive RR intervals (RMSSD) were used for analysis [2]. Furthermore, spectral powers in the low frequency (P_{LF}) band (0.04-0.15 Hz), high frequency (P_{HF}) band (0.15-0.40 Hz), their ratio (P_{LF}/P_{HF}) and total power (TP) were analyzed [22]. Power spectral density (PSD) was obtained using Welch's method [23].

2) Spectral entropy: Spectral entropy (SE) was calculated based on VLF (0.003-0.04 Hz), LF and HF powers as follows [24]:

$$SE = -P_{VLF} \times log_2(P_{VLF}) - P_{LF} \times log_2(P_{LF}) - P_{HF} \times log_2(P_{HF}) \quad (1)$$

3) Poincaré plot indices: The Poincaré plot provides an intuitive display of the dynamic properties of a system from time series. It is characterized by two indices; the width (SD1) and the length (SD2) of the plot which represent long and short-term variability of the nonlinear dynamic system [6]. SD1, SD2 and the ratio SD1/SD2 of the RR intervals were calculated for each subject.

4) Tone-entropy analysis: The difference of the consecutive RR intervals ($RR \equiv (RR_1, RR_2, ..., RR_N)$) corresponds to heart rate acceleration or inhibition. The percentage index (PI) was calculated as the percentage of the difference of two successive RR intervals divided by the first one and is positive for accelerations ($PI > 0$) and negative for inhibitions ($PI < 0$). Tone was derived from the first order moment of the (PI) and entropy was calculated from the probability distribution of PI, through Shannon's formula [25] to evaluate the total variations of heart period.

F. Statistical analysis

Results were expressed as median, 25% and 75% quartiles. A non-Gaussian distribution of the variables was found by the Chi-square goodness-of-fit test, the Kruskal-Wallis (KW) test was employed as a non-parametric statistical analysis method to compare HRV features across groups. The p-value of less than 0.05 was assumed significant. Mann-Whitney-Wilcoxon (MWW) was used as the non-parametric post-hoc test for pairwise comparison of HRV features between groups.

III. RESULTS

The KW test was applied to the HRV features from the subjects in six and four groups. Mean RR, SDNN, RMSSD, SD1, SD2, LF power, total power, spectral entropy and entropy were significantly different ($p < 0.05$) across six groups. Only SDNN, RMSSD, SD1, SD2, total power and entropy were significantly different across the four groups, while mean RR, LF power and spectral entropy were no longer different when I/D and I/I groups were combined.

TABLE II

P-VALUES OF KRUSKAL-WALLIS TEST ON VARIOUS HRV FEATURES COMPARED FOR SIX AND FOUR GROUPS.

HRV feature	Six group comparison	Four group comparison
Mean RR	0.0231*	0.1175
SDNN	0.0170*	0.0439*
RMSSD	0.0436*	0.0444*
SD1	0.0436*	0.0444*
SD2	0.0168*	0.0452*
LF power	0.0206*	0.0802
Total power	0.0041*	0.0131*
Spectral entropy	0.0206*	0.0802
entropy	0.0283*	0.0068*

The significant p-values $p < 0.05$ are marked by *.

As shown in table II, except for entropy, the p-values were smaller for six groups compared to 4 groups.

Results of the post-hoc tests for the significant features are shown in figure 1. According to four group pairwise comparison for SDNN, RMSSD and total power, the difference between T2D and control groups was significant for the combined I/D and I/I ACE genotypes. Pairwise comparison for Poincaré indices shows that the I/I group with T2D was significantly smaller than the indices of other groups but these differences were masked when this group was combined with the I/D group. Overall based on the all HRV features shown in figure 1, except for entropy, the I/I group with T2D was the most significantly different from the other genotype groups stratified by T2D and control.

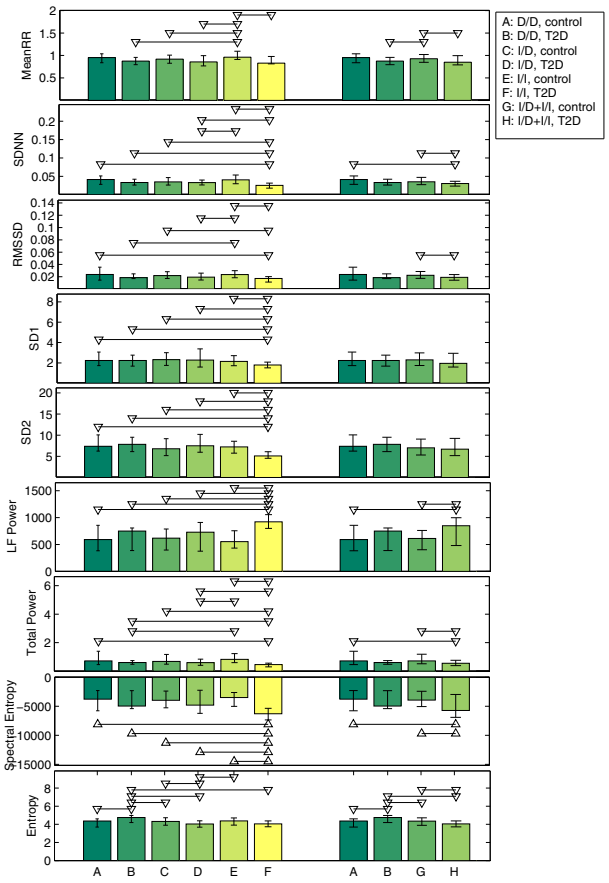

Fig. 1. Median, 25% and 75% quartiles of different significant HRV features for 6 groups and 4 groups. The significantly different pairs are marked by triangular pointers.

IV. DISCUSSION

Results of comparing HRV for different ACE genotypes in conjunction with diabetic status indicate that both can affect HRV and it is necessary to consider them together for analysis. For example Poincaré indices, LF power and spectral entropy of the group with I/I ACE genotype and T2D was different from all other groups, while the group with I/I ACE genotype but control, or other ACE polymorphisms plus T2D groups were not significantly different.

SDNN and RMSSD were found to be significantly different between D/D and combined I/D and I/I groups in an earlier study [14]. The results of the current study uncovered these differences in more detail by separating I/I and I/D and considering the diabetes status. For example SDNN of the I/I group with T2D was different from the I/D group with T2D, and this difference cannot be shown if these two groups are combined. Furthermore the SDNN of the D/D group is different from the I/I group only in conjunction with T2D, which indicates the importance of considering the diabetic status.

Different from the previous study by Busjahn et al. [14], the HF power of HRV was not significantly different across the groups. One of the differences of that study with the current one is the age range, which was 34±14 and 33±14 for the different groups in Busjahn's study, while it ranged from 65±10 to 70±7 for the groups in the current paper. According to a study by Stein et al. HF is significantly different for these different age groups and thus our older group may have masked the effect of genotype or diabetes status [26]. Another difference of the current study with previous ones is that automated preprocessing is used while manual corrections were performed in earlier studies. Preprocessing can also affect the HRV results as shown in our previous studies but automated preprocessing allows a more robust comparison between studies as the process is repeatable unlike when using subjective ectopic beat removal, which has a tendency for intra and inter-rater differences [17].

V. CONCLUSION

In this paper HRV was analyzed for individuals with different ACE polymorphism and diabetic status. Results indicated that both diabetes and ACE genotypes affect HRV features including: mean RR, SDNN, RMSSD, Poincaré indices, LF power, total power, entropy and spectral entropy. Therefore it is necessary to consider both factors together for HRV analysis. Comparison of I/I and I/D groups provides more details compared to analysis of the combined group. The I/I

group with T2D had the most different HRV compared to others and the differences of Poincaré indices of this group with others are masked when it is combined with I/D.

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