THE INFLUENCE OF AGE AND GENDER ON HEART RATE VARIABILITY

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Biomedical Engineering

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September 2020

DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

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ABSTRACT

Heart rate variability (HRV) has been recognised as a trait marker of mortality and morbidity vulnerability. However, its predictive value might have limited by the indefinite philosophies behind the physiological factors especially the age-related and gender-dependent changes over the life span. Prior works on HRV based on a comparative small population, were insufficient to define the impacts of age and gender on HRV. In this regard, this study attempted a clarification on the age and gender effects on HRV with a large sample size. A total of 14399 patients of which 7275 men and 7124 women who averagely aged 62.53 ± 17.28 years old were recruited in this study at Central Cardiology Sydney practice within a 10 year period. Gender and aging effects on HRV were evaluated by means of four time-domain HRV measures determined by SDNN, SDANN, SDNN-i and rMSSD which were extracted from 24-hour ambulatory ECG recordings that were routinely performed in subjects using 3-channel electrograph. It has been evidenced that, gender effects on HRV were significant ($p < 0.001$). All HRV measures were significantly depressed with aging ($p < 0.001$) except for rMSSD in which, a U-shaped curve was yielded ($p < 0.001$). The aging effects on all HRV measures were gender-dependent ($p < 0.001$) except HRV determined by rMSSD ($p = 0.631$). Overall HRV indexes were found to be statistically higher in male subjects compared to the age-matched female subjects. A linear correlated pattern of decrease with aging was observed in SDNN, SDANN and SDNN-i, whereas rMSSD demonstrated a quadratic term that yielded a U-shaped pattern for both genders. In short, a significant deterioration of cardiac autonomic regulation is common in healthy aging. Men excel women at cardiac autonomic regulation which the former are mainly vagal-modulated whereas the latter are primarily sympathetically-driven. A sudden improvement in the global autonomic regulation in women at the sixth decade could have reflected the substantial role of estrogen during womanhood. A more gradual reduction found in all HRV parameters and a progressive increase of rMSSD at the later age could have attributed to the greater longevity in women.

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LIST OF SYMBOLS / ABBREVIATIONS

ANS	Autonomic Nervous System
ANOVA	Analysis of Variance
ApEn	Approximate Entropy
CD	Correlation Dimension
DFA	Detrended Fluctuation Analysis
ECG	Electrocardiogram
FFT	Fast Fourier Transform
HF	High Frequency
HRV	Heart Rate Variability
IBI	Inter-beat Interval
LF	Low Frequency
LF/HF	Low Frequency to High Frequency Ratio
NN	Normal-to-Normal
NN50	Number of paired NN intervals which show a variations above 50 ms over the entire recordings
pNN50	Ratio of the NN50 count to the total NN intervals
PPG	Photoplethysmography
Q-Q	Quantile-to-Quantile
rMSSD	Root-mean-square of the Successive NN Intervals Difference.
SD	Standard Deviation
SDANN	Standard Deviation of Sequential 5-minute NN Intervals
SDNN	Standard Deviation of all NN intervals over 24 hours
SDNN-i	Mean of Standard Deviation of total NN intervals for all 5-min duration
TINN	Triangular interpolation of NN interval Histogram
ULF	Ultra-low Frequency
VLF	Very-low Frequency

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Cardiovascular disease is currently ranked as the first leading cause accountable for worldwide mortality, leading to approximately 17.9 million deaths per annum (World Health Organization, 2020). Therefore, early-stage cardiac irregularities diagnosis associated with an appropriate treatment is substantial. Indeed, there are many factors that caused deterioration of cardiovascular system. Of these, autonomic dysfunction might be one of them. It has been clearly shown in many studies that autonomic dysfunction is highly correlated with a diverse of illnesses, regardless of mentally or physically. A healthy heart does not beat systematically as a metronome. In fact, heart rhythm varies with every single beat. These variations measured in milliseconds between consecutive R peaks result in computation of heart rate variability (HRV) which in turn, mainly subjected to the extrinsic regulation of heart rate (Acharya *et al.,* 2006).

To unmask the predictive utility of HRV, it is essential to understand how heart rate is being controlled. Heart beat is predominantly driven by the sino-atrial (SA) node, by which the electrical impulses generated by the SA node will eventually evoke the atria contraction. SA node is in turn, regulated by both sympathetic and parasympathetic tone which are within the extent of autonomic nervous system. A notable increase and decrease of heart rate is associated with high level of sympathetic and parasympathetic activities respectively. Changes between successive heart beats are parasympathetically-mediated (Spiers *et al.,* 1993). This has been further proven in the study of Bootsma *et al.* (2003) in which, the HRV is not significantly correlated with sympathetic tone as all the correlations obtained were below 0.31. The integrity and complexity of heart are primarily modulated by the sympathovagal balance (Acharya *et al.,* 2006). Heart rate of a healthy individual is subjected to the physiological phenomenon, known as respiratory sinus arrhythmia under resting condition (Spiers *et al.,* 1993). This vagal-mediated event is intended to describe how the heart acts simultaneously with

respiration in which, a significant increase of heart rate is observed during inspiration whilst the opposite case for expiration. Cardio-acceleration and cardio-deceleration that are generated by the different respiration phases, provide insights into how well the ANS regulate the heart rate.

Evaluation of HRV can be performed via linear and non-linear methods (Malik *et al.,* 1996). Linear approaches include time-domain, frequency-domain and geometrical HRV analysis whereas the non-linear HRV analysis is conducted based on measures, take correlation dimension (CD), for instance. Variability in heart rate can be monitored via ultra-short-term (usually less than 5 min), short-term (within 24 hours, 5 min in particular) and long-term (more than 24 hours) ECG recordings. (Malik *et al.,* 1996; Acharya *et al.,* 2006; Shaffer and Ginsberg, 2017; Pecchia *et al.,* 2018). Of these, subjects' 24-hour HRV by means of four customary time-domain indexes which are the standard deviation of entire normal-to-normal (NN) intervals within 24 hours (SDNN), standard deviation of the means of all sequential 5-min NN intervals (SDANN), mean of standard deviation of the all sequential 5-min NN intervals (SDNN index) and root-mean-square of successive NN intervals difference (rMSSD) are being the interests of the study.

Indeed, HRV is proved to be clinically significant in reflecting the capability of heart when perceiving and countering to a sudden change of the incoming stimuli (Acharya *et al.,* 2006). In addition, it has been acknowledged to be used as a predictor in evaluating the clinical condition of ANS accountable for regulating cardiac activities. Up until recently, HRV analysis has been increasingly utilized to assess the autonomic nervous system (ANS) activity in a wide variety of diseases. These include those cardiac and non-cardiac diseases such as myocardial infarction (Carney *et al.,* 2001; Stein et al., 2005); diabetes (Kudat *et al.,* 2006); renal dysfunction (Drawz *et al.,* 2013; Oliveira *et al.,* 2014) and depression (Hartmann *et al.,* 2019). The indicative efficacy of HRV in clinical practice must take into account the effect of a wide variety of physiological factors such as age, gender and genetics; lifestyle factors which include the endurance activities and sedentary practice (Sammito and Böckelmann, 2016). Among these, age and gender are studied thoroughly as being the key determinants of cardiac autonomic nervous regulation (Umetani *et al.,* 1998; Vallejo *et al.,* 2005).

1.2 Importance of the Study

To date, HRV analysis has been widely accepted and adopted as a touchstone of the ANS performance. Variations between consecutive R peaks, particularly those derived from long-term ambulatory cardiac monitor-based have been extensively utilized to unmask the underlying causes of various cardiac and non-cardiac disorders. Although there are findings that focused on the physiological response associated with the factors such as age and gender with reference to the 24 hours long-term ECG recording, the data currently available to be employed for prognostic intention are mostly extracted from short-term based researches with relatively small sample size. Hence, the impacts of age and gender on 24 hours HRV indexes based on large sample size ($n > 10000$) will be statistically studied using time-domain analysis in this research project, with the assistance of software Statistical Package for the Social Sciences (SPSS) to provide more valuable knowledge for diagnosis and prognosis purpose.

1.3 Problem Statement

Most recently, evaluation of HRV has been increasingly utilized to unmask the philosophy behind the ANS activity in different diseases or various conditions owing to the comparative simplicity and relative great prognostic power. Lower HRV has been found to be strongly correlated with the increasing risk to morbidity and mortality (Malik *et al.*, 1996; Umetani *et al.*, 1998; Zulfiqar *et al.*, 2010). However, the clinical significance of HRV has cause controversy due to the uncertain mechanisms that drive the age-related and gender-associated physiological changes on HRV throughout the lifetime. Current researches on HRV that are mainly focused on a relative small sample size ($n < 10000$) are inadequate to provide a comprehensive understanding on aging and gender effects on HRV. Therefore, a study on the potential effects of demographic data ($n > 10000$) on HRV is necessary, particularly the impacts of age and gender on HRV are extensive to provide relevant information for clinical intervention.

1.4 Aim and Objectives

This research project is intended to statistically justify the influence of age and gender on heart rate variability. In order to attain the aim of the study, three objectives have been outlined, referred as follows:

a. To investigate the effect of age on HRV measured in 24 hours using time-domain method;
b. To determine the influence of gender on HRV measured in 24 hours using time-domain approach;
c. To study the gender difference with increasing of age on HRV measured in 24 hours using time-domain analysis.

1.5 Scope and Limitation of the Study

This research project sought to delineate the factors affecting HRV and their respective effects on HRV, the age and gender in particular. The effects on HRV in respect of age and gender were evaluated based on the appropriate statistical tests selected. Lastly, a final thesis that documented the development, flow and performance of the statistical analysis is presented in this report.

There are a variety of concerns deserved considerations and perhaps, the information about sleep such as the quality and duration are one of the caveats that should be taken into account. Previous studies have reported that the individuals especially those who are involved in the 24-hour ECG recordings, are required to undergo simultaneous polysomnography to record the sleep patterns, suggesting that the variations in sleep stages with age may have altered the autonomic nervous activity and thus, showed effects on HRV (Bonnemeier *et al.*, 2003; Beckers, Verheyden and Aubert, 2006). In this research, the text field data used to perform statistical analysis were collected without providing the details about the sleep, limiting the interpretation of circadian patterns owing to the insufficient information accountable for evaluation of HRV during nighttime. Thus, study of circadian variations in HRV was not carried out in this study.

It should be noted that, 24-hour HRV may varied according to different physical activity levels (Umetani *et al.*, 1998; Bonnemeier *et al.*, 2003; Sandercock, Bromley and Brodie, 2005; Beckers, Verheyden and Aubert, 2006). Thus, it is necessary to record the activity levels for all the subjects

involved by routinely monitoring their physical activities in order to prevent the misinterpretation of results. This can be accomplished by preparing wearable activity trackers for subjects that are lightweight, portable and user-friendly. However, the data were provided without the records of activity log over 24-hour period, indicating that an assumption can be made in which, the significant depression in HRV with aging may have resulted from the decreases in physical activity level. Aside from age-associated decline in HRV, it is further suggested that there is the possibility that having different levels of activity may have induced a higher HRV of men compared to that of age-matched women.

Needless to say, subjects' medical background is one of the considerations that should be taken into accounts in order to accurately analyse the effects on HRV in a more detailed manner. As outlined by previous findings, apart from the physiological variables such as age and gender, health issues such as BMI (Poirier et al., 2003; Vallejo *et al.*, 2005) and diseases concerning both cardiac and non-cardiac aspects (Cowan *et al.*, 1994; Kallio *et al.*, 2002) would have affected the HRV. Of these, researchers have discovered that individuals with myocardial infarction (Carney *et al.*, 2001; Stein et *al.*, 2005), diabetes (Kudat *et al.*, 2006), renal dysfunction (Drawz *et al.*, 2013; Oliveira *et al.* 2014) and depression (Hartmann *et al.*, 2019) would have significant lower HRV, unveiling the fact of the predominance of sympathetic tone in the patients who suffered these disorders. Thus, it is strongly recommended that the researchers should have gained a certain level of understanding into studied subjects before interpreting the data. Since the subjects were recruited without knowing their medical histories except their age and cardiovascular profile, it therefore cannot be simply excluded or concluded that the significant decrease or increase in each HRV measure is caused by the possible factors mentioned above.

1.6 Contribution of the Study

This study will redound to the benefit of society considering that HRV serves an important diagnostic tool in examining the condition of autonomic nervous system today. The findings may contributed to the predictive value of HRV by providing further explication with respect of changes on HRV that related to

age and gender across the lifetime. Besides that, the study may help to uncover critical areas in the diagnostic and prognostic process that many researchers were not able to explore as prior works which focused on small population were barely sufficient regard for the clarification on age-related and gender-dependent effects on HRV for the entire life.

1.7 Outline of the Report

This study composed of five chapters in total.

Chapter 1 introduces the research by briefly outlining the background of HRV, the importance of study, problem statement, aims and objectives, the scope and limitations and the contribution of study.

Chapter 2 represents the critical review on HRV in which, the measurements of HRV, the methods to analyse HRV, the period of ECG recordings, the clinical significance of HRV and the possible factors that could have affected HRV are included within this chapter.

Chapter 3 describes the methodology of the study. The research questions are included, followed by the incorporation of hypothesis and p-value. Various methods to test the data normality and ways to correct data skewness are presented within this chapter. Procedures that lead to a proper choice of statistical analysis are likewise demonstrated.

Chapter 4 outlines the results and discussions of the study. The results shown were normalized and illustrated in terms of tables, graphs and scatter plots. Age-HRV relations, gender-HRV associations and effects on HRV with maturity for both males and females are discussed.

Chapter 5 concludes the study by providing a brief summary of the study and offering few suggestions for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Heart rate variability, as abbreviated as HRV, is the measurement of successive heartbeat variations within a specific timeframe, particularly measured in milliseconds (ms). It is thought to reflect the state of an individual, concerns with his or her physical fitness and corporeal preparedness especially when unexpected inputs were detected. HRV is considered low when the heart beats relatively fast, indicates that the subject was either partaking in vigorous exercises or experiencing psychological stress. On the contrary, higher HRV demonstrates that the body is trying to restore back the homeostasis from the thrilled phase by slowing down the heart rate and increasing the HRV. It is interesting to disclose the fact that there was a conflict in determining the HRV score is either a ‘good’ or ‘bad’. Due to its characteristic of being highly individualized, it is necessary to take into account the potential physiological mediators such as age and gender, as these factors were significant to the clinical analysis and assessment of HRV in order to gain a better perspective in diagnosing and treating different diseases, particularly those associated with autonomic nervous system.

2.2 Measurement of Heart Rate Variability

Generally, HRV can be obtained by either via consecutive R peaks (RR interval) of the QRS complex indicated by a normal heartbeat or sometimes, acquired by computing the inter-beat interval (IBI).

2.2.1 RR Interval

RR interval can sometimes called as normal-to-normal (NN) interval, but this only bona fide when the intervals are derived from the normal R peaks which R peaks from ectopic heartbeat must be excluded. It is noted that not all the RR interval can be NN interval, but all NN interval must be RR interval. Acquisition of successive RR interval is generally computed by interpreting the electrocardiography (ECG) that is represented in a graphical manner. ECG

indicates the cardiac activity over a period of time. Cardiac cycle of healthy individual is constituted of a standard PQRST waveform. P wave corresponds to the atrial depolarization which in turn, initiated by the pacemaker cells in the sinoatrial node and led to atrial contraction or atrial systole. For a standard ECG, QRS complex succeeds P waves. P wave is characterized by its appearance as a small hump projected from the baseline which is usually smooth and round. PR interval commences with depolarization of atria whilst ceases with beginning of the depolarization of ventricle. It is often used to indicate whether the atrioventricular (AV) node is blocked which eventually lead to atrial enlargement.

QRS complex indicates the ventricular depolarization, with the notable high amplitude when compared to P and T waves due to the larger tissue mass of ventricular. It is initiating with a negative deflection found in Q section, then a significant positive deflection is noticeable for R segment, followed by a return to the baseline of S portion. ST segment came into play to connect the QRS complex and T wave. Rapid repolarization of ventricle indicates the event of T wave. PQRST complex forms the substantial part of ECG, followed by U wave which in turn, accountable for atrial repolarization. However, its presence is usually ignored as it cannot be clearly noticed unless a specialized electrode is used to detect its existence.

The frequency of ECG signal is typically within the range of 0.5 Hz to 100 Hz, chiefly rely on the application. According to Tereshchenko and Josephson (2015), P wave is identified with frequencies lie within a range from 5 Hz to 30Hz. On the other hand, QRS complex often settles within the frequencies of 8 Hz to 50 Hz. Frequencies of 0 (DC) to 10 Hz characterize the T wave. An extremely high frequency, normally above 70 Hz is noted when unusual ventricular conduction occurred. Amplitudes of ECG signals are often represented in millivolts (mV) and the intervals of each segment are expressed in milliseconds (ms). These information can be collected by analysing and interpreting the graph that is plotted on the ECG paper. HRV derived from ECG has been proven to be more reliable owing to capability of ECG in providing an overview into the physiological status of cardiac health and its relatively high stability even during vigorous exercise. A standard ECG signal

is illustrated as Figure 2.1 (Mathworks.com., 2020). Table 2.1 summarized the amplitudes, frequencies and intervals of the classic ECG waveform.

Table 2.1 Amplitudes, Frequencies and Intervals of ECG Signal

Feature	Amplitude (mV)	Frequency (Hz)	Interval (ms)
P wave	0.05 to 0.25	5 – 30	< 80
QRS complex	• Greater than 0.5mV in at least one limb leads • Amplitude of Q wave is one-third of the R wave	8 – 50	80 – 100
T wave	• Less than 5 millivolts is obtained for precordial leads • Less than 10 millivolts is observed for limb leads	0 – 10	160

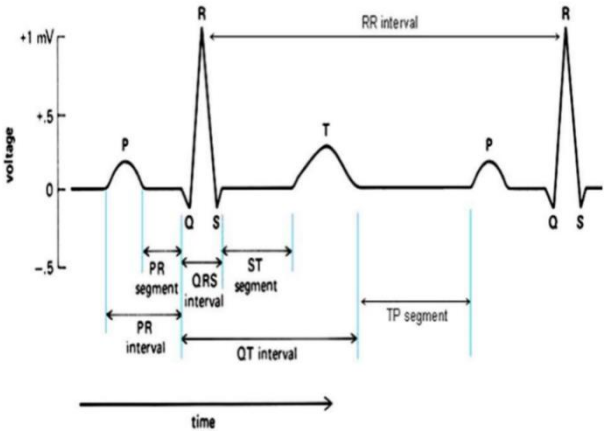


Figure 2.1: Real-time ECG QRS Detection

Successive RR intervals can be acquired via conventional ECG. The ECG signal is generally obtained using either standard 12-lead ECG or Einthoven's triangle. The standard 12-lead ECG is conducted by attaching 10 skin electrodes on the subject. Outcome is predominantly dependent on the 12 views acquired from different perspective angles. The electrodes are placed on the specified sites that are being standardized on the body surface. Acquisition of ECG signals is done by continuously recording the potential differences of

myocardial cells during the events of depolarization and repolarization for every single cardiac cycle. Placements of electrodes are clearly manifested in the Table 2.2 (Randazoo, 2016) and Figure 2.2 (Randazoo, 2016).

Table 2.2: Placement of 12-Lead Electrodes

Electrode	Placement
V1	4th Intercostal space to the right of the breastbone
V2	4th Intercostal space to the left of the breastbone
V3	Midway between V2 and V4
V4	5th Intercostal space at the midclavicular line
V5	Anterior axillary line at the same level as V4
V6	Midaxillary line at the same level as V4 and V5
RL	Anywhere above the right ankle and below the torso
RA	Anywhere between the right shoulder and the wrist
LL	Anywhere above the left ankle and below the torso
LA	Anywhere between the left shoulder and the wrist

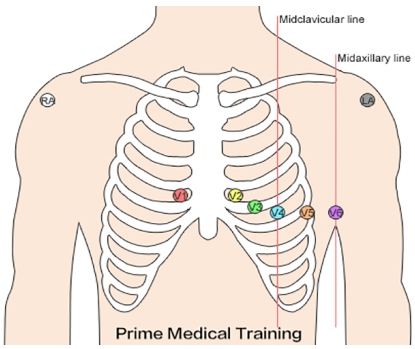


Figure 2.2: 12-Lead ECG Placement

In addition to the 12-lead ECG, Einthoven's triangle is believed to be one of the worthwhile interventions to acquire the ECG signals (Jin *et al.*, 2012). Einthoven's triangle is first discovered and invented by Willem Einthoven (Conover, 2003). It is also known as bipolar recordings. It is basically employed in electrocardiography by forming an imaginary inverted equilateral triangle. Triangle is constructed in such the way that placing the leads on two shoulders and the pubis with the heart at the centre (Williams and Wilkins, 2010). State of cardiovascular system is reflected by measuring the electric fields due to the events of depolarization and repolarization. Electric fields are generated by heart and often represented in form of vector, can be

acquired by tracking the potential differences between the electrodes (Richardson, Randall and Speck, 1998). Apart from being competent in providing the useful information regarding to cardiac activities, Einthoven's triangle is able to contribute in the aspect of lead misplacement. Identification of proper lead placement is vital to provide appropriate diagnosis and treatment by avoiding the possible mistake caused by the misrepresentation of the ECG obtained due to incorrect lead placement. The equilateral triangle is formed by placing three bipolar limb leads. Each of the lead will create an axis that measure the potential differences by placing the positive and negative electrodes on the prescribed site, referring to Table 2.3 (Thaler, 2003) and Figure 2.3 (Williams and Wilkins, 2010).

Table 2.3 Lead Placement of Einthoven's Triangle

Lead	Description	Potential Difference	Degree of Orientation
I	• Form an axis from left arm to right arm • Left arm lead as positive electrode • Right arm lead as negative electrode	I = LA – RA	0
II	• Form an axis from right arm to left leg • Left leg lead as positive electrode • Right arm lead as negative electrode	II = LL – RA	+60
III	• Form an axis from left arm to left leg • Left leg lead as positive electrode • Left arm lead as negative electrode	III = LL - LA	+120

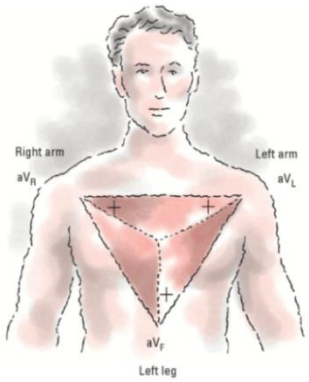


Figure 2.3: Lead Placement of Einthoven's Triangle

2.2.2 Inter-beat Interval (IBI)

Inter-beat interval, sometimes could be written as interbeat interval or expressed as IBI, is used to describe beat-to-beat interval that measured in milliseconds (ms) which is comparable to the RR interval that being implemented to compute the HRV. IBI, as illustrated in Figure 2.4 (Lee and Chung, 2012), is measured based on the application of the principle of photoplethysmography (PPG) via typical pulse oximeter. A conventional pulse oximeter consists of a pulse sensor, which is normally attached to subject's body surface. The attachment sites can be varied, but perhaps the most commonly used finger, forehead and earlobe.

The change in the blood flow volume through any organ of the subject's body results in the variation in light intensity across the vascularized parts as the blood itself absorbs a certain amount of light. The sensor itself is generally equipped with a light-emitting diode (LED) and a detector. When the sensor is attached to prescribed site, the tissue irradiated with a light source will leads to a definite amount of reflection and the reflected light will then be detected by the light sensor. The total light reflected are mainly dependent on the amount of blood flow volume which in turns hinges on the heartbeat of the subject. Reflected light is outputted by detector as electrical signal. The resulted electrical signal is thus corresponding to the pulse rate.

Lu *et al.* (2009) conducted an analysis to compare the HRV signals extracted from PPG and ECG, suggesting that PPG is a practicable alternative to ECG for the extraction of HRV signals. However, the clinical practicability of PPG has been much disputed. In the study of Jan *et al.* (2019), 30 subjects were recruited and the respective ECG and PPG signals were measured in the

course of spontaneous and controlled breathing. The results showed that the ECG-derived HRV parameters were significantly corresponded with breathing than PPG-computed ones. It is thus suggested that PPG might not capable to be utilized as a meticulous screening tool for heart beat detection, the cardiopulmonary analysis for controlled breathing manoeuvre in particular.

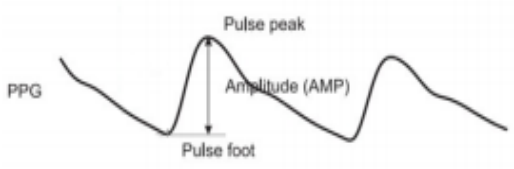


Figure 2.4: Inter-beat Interval (IBI)

2.3 Heart Rate Variability Analysis

Assessment of HRV has been standardized within the European Heart Journal, by the Task Force in year 1996. Variations in heart rate can be quantified by applying either linear or non-linear method, in accordance with the Task Force Report (Malik *et al.,* 1996).

2.3.1 Linear Method

There are few methods to analyse variations in heart rhythm linearly include time-domain analysis which is demonstrated either in statistical or geometrical representation; frequency-domain assessment which is also known as spectral-based analysis; and lastly, the nonlinear method which is chiefly used to appraise the complexity of sinus rhythm accountable for hemodynamic fluctuations (Malik *et al.,* 1996; Kleiger *et al.,* 2005; Shaffer and Ginsberg, 2017).

2.3.1.1 Statistical Analysis

According to the Task Force Report, statistical analysis can be performed by directly assessing successive R peaks intervals or evaluating the variations between the normal sinus RR interval or also known as NN interval. Time-domain variables should be taken into account when conducting statistical assessment in order to evaluate the relation between HRV and sympathovagal balance. Standard deviation of all NN intervals (SDNN) is commonly computed over 24 hours. SDNN is comparable to total power in the spectral

analysis. Thus, SDNN is capable to reflect the overall cardiac autonomic regulation based on a comprehensive evaluation of cyclic components accountable for variations in heart rate (Malik *et al.*, 1996).

In addition to the incorporation of SDNN, standard deviation of average NN intervals (SDANN) is frequently used in which, the standard deviation of the means of all sequential 5-min NN intervals is computed. SDANN is intended to evaluate the heart rate alterations owing to the lengthy cardiac cycles (usually more than 5 minutes). Average of standard deviation of the overall NN intervals in each five minute duration over 24 hours (SDNN index or SDNN-i), is well-appreciated to be used in quantifying the variations in heart rhythm ascribed to short cardiac cycles (commonly less than 5 minutes) (Malik *et al.*, 1996).

Differences between sequential normal R peaks devoted to the computation of variables to be statistically used to assess vagal modulation. These include the commonly used root-mean-square of NN interval differences in succession (rMSSD), number of paired NN intervals which manifest variations above 50 ms over the entire recordings (NN50 count) that measured in milliseconds (ms) and ratio of the NN50 count to the total NN intervals (pNN50) that is normally expressed in the form of percentage (%). These variables derived from the variations between consecutive normal heartbeats are strongly corresponded with high frequency power of the frequency-domain analysis, thus denoted a solid mutual relationship among each other as specified by the Task Force Report (Malik *et al.*, 1996). Table 2.4 summarized the formula for each index.

Table 2.4: Formula for Time-domain HRV Indexes

HRV Index	Formula	Units
Mean	$\frac{1}{N}\sum_{i=1}^{N} RR_i$	ms
SDNN	$\sqrt{\frac{1}{N}\sum_{i=1}^{N}(RR_i - \overline{RR})}$	ms
SDANN	$\sqrt{\frac{\sum_{i=1}^{288}(\overline{RR_i} - \overline{RR_{5min}})^2}{288}}$	ms
SDNN-i	$\frac{1}{N}\sum_{i=N}^{N} SDNN_i$	ms
rMSSD	$\sqrt{\frac{\sum_{i=1}^{N-1}(RR_i - RR_{i+1})^2}{N-1}}$	ms
NN50 count	$\sum_{i=1}^{N}\{\lvert RR_{i+1} - RR_i\rvert > 50ms\}$	-
pNN50	$\frac{NN50}{N} \times 100$	%

2.3.1.2 Geometrical Approach

Heart rate variability (HRV) can be analysed by transforming the adjacent NN intervals into geometrical patterns. Dimensionless HRV triangular index is widely-practiced as one of the geometric methods that illustrated HRV as the proportion of sum of intervals between adjacent R peaks and stature of RR interval histogram. On the other hand, TINN, the abbreviation of triangular interpolation of NN interval histogram, is computed according to baseline breadth of the triangular normal R peaks intervals histogram and usually measured in milliseconds (ms) (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017). Geometrical approaches are ideal for HRV evaluation as they are comparatively independence of the quality of intervals of R peaks (Malik *et al.*, 1993; Malik *et al.*, 1996). However, complication emerged due to insufficient practicable RR intervals to form the geometrical pattern (Malik *et al.*, 1996). Figure 2.5 illustrated the both geometric methods for HRV (Metelka, 2014).

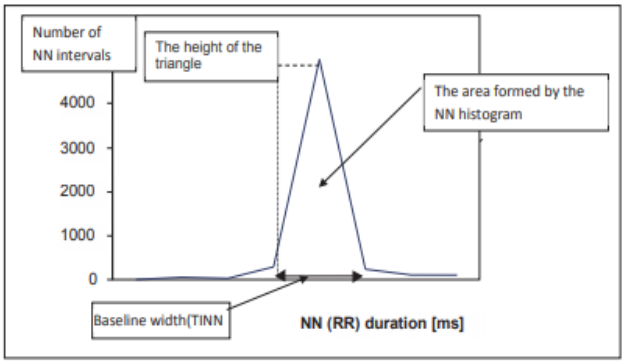


Figure 2.5: Geometric Methods for HRV Measurement

2.3.1.3 Frequency-domain

Fourier analysis, also known as spectral or frequency-domain analysis, is one of the linear methods that used to analyse HRV (Malik *et al.,* 1996). Changes of heart rates in different successions measured in time series can be expressed in a function of frequency. There are few ways to transform time-based measures include both parametric and non-parametric methods, but perhaps the most frequently employed is the non-parametric Fast Fourier Transform (FFT) owing to the simplicity of the algorithm (Malik *et al.,* 1996). Results obtained are then used for the computation of power spectral density in which, frequency distribution of power can be determined respectively. In the Task Force Report (Malik *et al.,* 1996), spectral analysis contributes an overview into physiological states of cardiac sympathovagal modulation by looking into five spectral components which are discussed as below.

Ultra-low frequency (ULF) power, which is characterized within the frequency range of 0 Hz to 0.003 Hz, has been found to be highly associated with SDANN (Shaffer and Ginsberg, 2017). Underlying cause of the development of ULF components remained unknown. Malik *et al.* (1996) and Shaffer, McCraty and Zerr (2014) demonstrated that the circadian rhythms are of paramount importance on the generation of ULF power. Regulatory processes that required a significant longer time to perform such as body temperature regulation and metabolism mechanism may be the largest contributor to the circadian oscillations in heartbeats which in turn, donated to the development of ULF power (Shaffer, McCraty and Zerr, 2014; Shaffer and Ginsberg, 2017).

Very-low frequency (VLF) power is fell underneath the frequency of 0.003 to 0.4 Hz. In order to best exploit the clinical practicability of VLF, a recording period length of at least 5 minutes is required (Malik *et al.,* 1996; Shaffer and Ginsberg, 2017). Hadase *et al.* (2004) carried out a frequency-domain analysis based on 54 chronic heart failure (CHF) patients, outlined that all the HRV frequency-domain variables decreased in patients with cardiac events, with the notable differences found in the VLF, low frequency and total power compared with healthy volunteers. Prognosis power of VLF in predicting cardiac event has been again evidenced using multivariate analysis. Apart from being influential marker in quantifying the risk to cardiac event, VLF component is strongly correlated with all-cause mortality compared to low frequency and high frequency component (Hadase *et al.,* 2004; Shaffer, McCraty and Zerr, 2014). However, the underlying physiological mechanism responsible for the generation of VLF band is remained ambiguous (Kleiger *et al.,* 2005; Shaffer and Ginsberg, 2017). Taylor *et al.* (1998) demonstrated that a significant decrease in parasympathetic activity could have generated a profound effect on VLF component in which, VLF power is found to be nearly diminished when there is a parasympathetic blockade. Contrary to what have proposed by Taylor *et al.* (1998), Shaffer, McCraty and Zerr (2014) and Shaffer and Ginsberg (2017) propounded that VLF power is predominantly dependent on the intrinsic heart rate. Efferent sympathetic activity is the major drive that governs the alterations of frequency and amplitude of these haemodynamic fluctuations. When there is a significant stressor such as physical activity, stress responses and other factors that increase efferent sympathetic activation, VLF power can cross over into the lower region of the low frequency band during ambulatory monitoring.

Low frequency (LF) power is particularly lay within the frequency band of 0.04 to 0.15 Hz. However, to date, there are conflicts owing to the uncertainty of autonomic regulation accountable for the assessment of LF band. In the early publications, LF component is suggested to be served as a marker of cardiac sympathetic outflow (Malliani *et al.,* 1991; Montano *et al.,* 2009). In contrary to the early works, Goldstein *et al.* (2011) and Rahman *et al.* (2011) postulated that the utility of LF power as an indicator of baroreflex function (Goldstein *et al.,* 2011; Rahman *et al.,* 2011). Reyes del Paso *et al.* (2013)

evaluated the suitability of HRV determined by LF power as an index of sympathetic cardiac control, suggesting that HRV power spectrum including LF component, is mainly determined by parasympathetic control. However, the study of Martelli *et al.* (2014) disagreed with the findings, demonstrated LF power is inappropriate to be applied as marker, either for cardiac sympathetic regulation or for baroreflex sensitivity. Shaffer, McCraty and Zerr (2014) suggested that LF power can be used to predict the regulation of heart rate in term of sympathetic control, exclusively for long-term recording. There are further studies proposed that low frequency power is mainly driven by both sympathetic and vagal tone (Shaffer and Ginsberg, 2017). Heart beats which are fell below the frequency of 0.1 Hz, are typically generated by sympathetic tone whilst the parasympathetic nervous system is particularly accountable for the heart rates within the range of 0.05 Hz (Shaffer and Ginsberg, 2017).

High frequency (HF) band is likewise known as respiratory band as it is highly corresponded with the variations of heart rhythm which is primarily driven by the respiratory mechanism, a phenomena called as respiratory sinus arrhythmia (Shaffer and Ginsberg, 2017). HF power is specified within the range of 0.15 Hz to 0.40 Hz, served as a prognosticator indicating the activity of parasympathetic nervous system (Malik *et al.*, 1996; Shaffer, McCraty and Zerr, 2014; Shaffer and Ginsberg, 2017). Autonomic nervous system can be evaluated by the fraction of LF power and HF power that is likewise abbreviated as LF/HF ratio. The proportion of LF to HF power is an attempt to explicate the sympathovagal balance. When a relatively high LF/HF ratio is observed, the sympathetic division is dominant which is usually accompanied by a notable cardio-acceleration. On the contrary, an individual is chiefly driven by parasympathetic activities when a low LF/HF ratio is obtained. (Malik *et al.*, 1996; Shaffer, McCraty and Zerr, 2014; Shaffer and Ginsberg, 2017).

Spectral analysis has advantage over time-domain evaluation as being easier to perform (Malik *et al.*, 1996). However, spectral-based approach is not preferable to be implemented in long-term HRV analysis due to the issue of uniformity. Physiological mechanism accountable for LF and HF power is considerably unstable along the recording period and a less definitive result is obtained (Malik *et al.*, 1996; Acharya *et al.*, 2006).

2.3.1.4 Correlation between Time-domain and Frequency-domain

According to Task Force Report, time-domain variables were mostly comparable to the spectral components. They were mathematically and physiologically equivalent when they were both measured via long-term recording (Malik *et al.,* 1996). Table 2.5 demonstrated the relation between time-domain and frequency-domain parameters over 24 hour recording duration (Malik *et al.,* 1996).

Table 2.5: Correlation between Time-domain and Frequency-domain

Time-domain Variable	Frequency-domain Correlate
HRV Triangular Index	Total power
TINN	Total power
SDNN	Total power
SDANN	ULF
SDNN-i	Means of 5 min total power
rMSSD	HF
NN50 count	HF
pNN50	HF

2.3.2 Non-linear Method

Non-linear approach is came into play when there is not a direct relationship between the variables measured. In a non-linear relationship, any alterations of the input do not directly yield a change in output. Non-linear parameters assess the complexity of the mechanisms responsible for HRV regulation (Shaffer and Ginsberg, 2017). According to the Task Force Report (Malik *et al.*, 1996), non-linear analysis provides an overview into the physiological interpretation of HRV as well as the vulnerability to sudden death. There are a number of non-linear parameters that are currently employed into the clinical practice. Of these, approximate entropy (ApEn), detrended fluctuation analysis (DFA) and correlation dimension (CD) are mostly found in the non-linear HRV analysis (Kallio *et al.,* 2002; Beckers, Verheyden and Aubert, 2006; RenuMadhavi and Ananth, 2010; Vandeput *et al.,* 2012; Voss *et al.*, 2013).

Approximate Entropy (ApEn) is used as a predictive tool to evaluate the complexity and regularity of the applied time-series data (Shaffer and Ginsberg, 2017). Computation of ApEn is mainly dependent on three measures which are the data length (N), the threshold (r) and lastly, the embedding dimension (m) (RenuMadhavi and Ananth, 2010). Data characteristic can be determined using ApEn values in which, high ApEn value demonstrate a random data that has low predictability of fluctuations whilst a deterministic data is associated with low ApEn value, indicating that the data is methodical and predictable (RenuMadhavi and Ananth, 2010; Shaffer and Ginsberg, 2017).

Detrended fluctuation analysis (DFA) is intended to assess the relationship between the variations of successive R peaks across different time spans. Slope α1 and α2 are resulted from the DFA and served as indicators for both brief and long-term fluctuations respectively. Short-term and long-term correlations that drawn out from DFA are thought to reflect the baroreceptor sensitivity and the underlying mechanism that control the fluctuation of the cardiac cycle (Shaffer and Ginsberg, 2017). Correlation dimension (CD) is designed to be used in the development of a dynamic model in which, the minimum number of variables required are determined. Complexity is mainly counting on the amount of variables encompasses to predict the time series (Shaffer and Ginsberg, 2017). Furthermore, high CD value is common in chaotic data. A low CD value is associated with a significant reduction of HRV and found in different cardiac disorders.

Non-linear methods have been frequently used to capture the system features that cannot be extracted from linear analysis using a more complex perspective (Müller, Jung and Ahammer, 2017). However, application of non-linear techniques has been limited owing to the computational complexity as the algorithms employed are relatively complicated. Computation of HRV measures under the non-linear practice will take up a longer time to execute, unlike the linear-methods which are normally done within milliseconds (Malik *et al.,* 1996; Müller, Jung and Ahammer, 2017).

2.4 Period of Electrocardiogram Recording

Period of recordings can be classified into three categories which are ultra-short-term (UST), short-term and long-term recordings respectively (Shaffer and Ginsberg, 2017). In the study of Shaffer and Ginsberg (2017), ultra-short-term recording is referred to any differences between heart rates that being recorded within the time, particularly less than 5 minutes (usually 1 to 2 minutes or even less than that). Alterations in heart rhythms can be particularly collected within 24 hours (5 minutes in particular) and the changes obtained under this practice are known as short-term heart rate variability (HRV). Changes in heart rates that being acquired more than 24 hours, are known as long-term HRV, sometimes also known as 24-hour HRV (Malik *et al.,* 1996; Shaffer and Ginsberg, 2017; Pecchia *et al.,* 2018).

Period length of ECG recording is predominantly dependent on the interest of the study. If the fluctuations in consecutive R peaks with cardiac cycles longer than 5 minutes were desired, it is necessary to consider the long-term ECG recordings which is usually carried out for 24 hours as all cyclic components of cardiovascular system could be accounted using long-term HRV (Kleiger *et al.,* 2005). Popularity of 24-hour recording has been evidenced with the increasing usage in many studies of HRV owing to its capability in providing high prognosis accuracy as HRV recorded under longer period able to provide an overview into the state of cardiac autonomic system with a greater exposure to the environment stimuli. In addition, 24-hour HRV values enable parties of interest to observe variations between daytime and nighttime which cannot be performed via short-term recordings (Kleiger *et al.,* 2005; Nunan *et al.,* 2010).

HRV recorded through short-term recording should be evaluated using frequency-domain instead of time-domain method. Frequency-domain based HRV measures are more efficient to reflect cardiac sympathovagal balance by providing more explainable results for short recording period. For long-term recording, time-domain method is desirable than frequency-domain. This is due to the results from frequency-domain method become difficult to interpret and less definite owing to increasing inconsistency resulted from fluctuations in heart rates for relatively longer recording duration (Malik *et al.,* 1996).

2.5 Clinical Significance of Heart Rate Variability

HRV assessment has been proved to be clinically significance in providing percipience into the integrity of cardiac autonomic modulation and the vulnerability to various disorders other than cardiac-related diseases. To date, HRV analysis have their placed for clinical evaluation, particularly in the field of cardiology such as myocardial infarction (Carney *et al.,* 2001; Stein et *al.,* 2005); psychology such as depression (Hartmann *et al.,* 2019); endocrinology such as diabetes (Kudat *et al.,* 2006) and nephrology such as chronic kidney disease (Drawz *et al.,* 2013; Oliveira *et al.* 2014).

2.5.1 Autonomic Nervous System

Autonomic nervous system (ANS) is one of two substantial divisions of peripheral nervous system (PNS) which is principally responsible for all physiological functions that are performed without conscious attempt. ANS modulates reflexive body processes by generating involuntary response such as blood pressure, heart rate, digestion, rate of inhalation and exhalation, urination and sexual reaction. ANS regulation is predominantly dependent on the coordination of hypothalamus that is located at a region of the forebrain below the thalamus. ANS can be further break down into three subdivisions which are sympathetic, parasympathetic and enteric nervous system. Of these, sympathetic and parasympathetic divisions are the two that should be paid attention to since enteric nervous system is only accountable for gastrointestinal system.

Autonomic nervous system is substantially modulated by a complex interplay of sympathetic and parasympathetic regulation. Sympathetic activities are significant when a person is under excitation states either psychologically or physically. Fight and flight mechanism is the modulator in which, trigger the activation of sympathetic nervous system, thus allow body to react to the acute stress or changing circumstances. The fight and flight response is initiated by release of catecholamines, typically the adrenaline and noradrenaline hormone which is originated from adrenal gland. During fight and flight response, rapid pulse rate and respiration rate are observed which aid the body to keep up with demand. Some physiological functions are temporary inhibited and the blood is diverted to skeletal muscle to defend

body against attack. Pupil dilation, flushed skin and sometimes, a slight tremble are the symbolic responses of fight and flight mechanism.

Parasympathetic nervous system is likewise called as rest and digest mechanism. It serves as a physiologically contrasting approach to the sympathetic nervous system. It complements the sympathetic division and predominantly dependent on the stimulation of vagus nerve, thus it is likewise termed as vagal nervous system. Parasympathetic tone governs when an individual feels relax under a cosy environment which puts the subject at ease. Parasympathetic dominates upon the activation of neurotransmitter acetylcholine, restore the body processes to the normal and exhibit digestion by enhancing blood flow towards gastrointestinal (GI) tract via dilated blood vessels and speeding up the peristalsis. When there is a significant higher level of parasympathetic activities, a swift heart rate is decelerated and returned to the standard magnitude. Significant rapid breathing rate is slowed down by bronchioles constriction as the high demand of oxygen has reduced.

2.5.2 Assessment of Autonomic Nervous System

To date, impairment of autonomic nervous system can be evaluated via various processes which have been evidenced to be effective and efficient for clinical intervention. Among these conventional indirect cardiovascular reflexes evaluations, four stand out as being of crucial importance: Valsalva manoeuvre, deep breathing, orthostatic and head-up tilt assessment.

Valsalva manoeuvre is performed to assess sympathetic adrenergic functions using blood pressure responses as well as cardiovagal functions using heart rate responses (Zygmunt and Stanczyk, 2010; Novak, 2011). In general, the whole process is divided into four phases in which, phase I is indicated by the occurrence of bradycardia in the first few seconds owing to the activation of baroreceptor caused by a gradual elevation in thoracic pressure, thus subsequently lead to a moderate improvement of stroke volume when the subject is assigned to exhale vigorously against a closed airway. Phase II demonstrated a notable rise in heart rate, which serves to counterbalance the insufficient venous return and low stroke volume, is observed during the 15 to 20 seconds of the manoeuvre which is characterized by the termination of respiratory actions by clamping or pinching one’s nose.

Clamp is released, denoted the onset of phase III. Aortic volume increased while the stroke volume continued to decrease, leading to further momentary drop in blood pressure accompanied by a further increment in heart rate. During phase IV, cardiac output is restored with a significant increase of stroke volume and blood pressure. Valsalva ratio is computed as a fraction of greatest consecutive R peaks difference in stage IV and smallest successive R peaks variation of stage II. A healthy subject should have a valsalva ratio value of 1.5 or higher (Singh *et al.*, 2016).

Deep breathing, also known as diaphragmatic breathing is one of parasympathetic measures that accounts the respiratory sinus arrhythmia (RSA) in which, provide insights into the degree of synchronization of HRV with respiration. Differences between adjacent R peaks tend to decrease during inspiration and increase during expiration. Subject is required to respire at the rate of 6 breaths/min in the sedentary statues. Variations in heart rate which is corresponding to the ventilation mechanism are recorded. RR intervals which are associated with inhalation and exhalation, devote to the computation of inspiratory-expiratory (I:E) ratio, which is represented as the ratio of the smallest variations of adjacent R peaks during inspiration to the greatest differences between consecutive R peaks during expiration (Zygmunt and Stanczyk, 2010). I:E ratio of 1:2 is regarded as normal and is formulated in accordance with the fact of longer expiratory duration is documented, approximately twice of the inspiratory time (Sembroski, 2018).

Orthostatic test is used to evaluate the alterations in heart rate when respond to active standing. Orthostatic parameter is obtained by asking subject to rest in a supine recumbent posture for 15 minutes at the beginning of the assessment. Subject is then required to stand up from the resting states and ECG readings are continuously recorded. A significant amount of blood rushed to the lower parts of body, which eventually leads to a global reduction of venous return and stroke volume due to the sudden change from supine posture to upright position. To recompense the significant loss of the cardiac output, compensatory tachycardia is activated, with concomitant plummet found in systolic and diastolic blood pressure for the first 30 seconds. This is followed by the phase of stabilization in which, the systolic and diastolic blood pressure decreased and increased respectively with a notable heart rate

escalation of 10-15 beats per minute. 30:15 ratio is used to assess the effort of vagal tone to return heart rate to the normal level which defined as a quotient of interlude between R peaks at 30^{th} beat and the variation of R peaks in succession at 15^{th} beat (Zygmunt and Stanczyk, 2010). 30:15 ratio demonstrates a reduction with maturity and normal value of at least 1.04 is reported in the finding of Zygmunt and Stanczyk (2010).

Head-up tilt test is conducted to unmask the temporary loss of consciousness caused by a fall in blood pressure level which is expressed as syncope in the medical term by evaluating one's reaction to orthostatic pressure. It is also known as tilt-table test as subject is asked to lie down on the table that can be swivelled up to a maximum of 90 degree, bound to the table using strap. Subject is then passively tilted to certain degree, usually 60-80 degrees (Zygmunt and Stanczyk, 2010). Continuously pulse rhythms and blood pressure monitoring are conducted throughout the test, which takes time approximately 20-60 minutes. During tilt-table test, administration of medicines such as nitrates, isoproterenol and clomipramine is necessary to reactivate the sympathetic division. Subjects who get a positive for tilt table test indicates the development of syncope associated with significant low blood pressure, consequently lead to the event of faint out or commonly known as passing out. A sudden faint out may have disclosed the underlying mechanisms of cardiovascular and autonomic nervous system. Cardiogenic syncope, particularly the vasovagal syncope, is resulted from a significant drop in blood pressure associated with a conspicuous fall in heart rate. This may unmask the physiological status of cardiac autonomic control to identify the risk to various cardiac disorders.

Study of O'Brien, O'Hare and Corrall (1987) manifested that autonomic dysfunction can be quantified via conventional cardiovascular reflex tests. 310 healthy subjects aged 18-85 years were recruited and the results demonstrated a significant decline of valsalva ratio with increasing of age. Since valsalva ratio is intended to evaluate the sensitivity of baroreflex receptors towards sudden changes, it also indicates the sympathovagal responses when baroreflex receptor is activated either due to hypotension or hypertension. Thus, a significant low valsalva ratio might signified an autonomic dysfunction. 309 healthy subjects with an average age of 36.7 ±

13.8 years were recruited in the study of Agelink *et al.* (2001) which demonstrated that I/E ratio and 30:15 ratio were correlated negatively with age. There is no significant gender difference found in 30:15 ratio. Gender differences were found in I/E ratio in which, men showed a higher I/E ratio when compared with women.

2.5.3 Heart Rate Variability and Autonomic Nervous System

Among the techniques used to assess the ANS, HRV analysis had emerged as a relative simple, non-invasive and easily applicable tool. Variations in heart rate is generally controlled by the ANS. Changes between heart beats are thought to reflect the adaptability of ANS to adjust and react to sudden incoming internal and external stimuli according to changing situation (Acharya *et al.,* 2006). Autonomic nervous system is believed to be functioned well by steadily adapting and responding to unexpected inputs if it is in sympathovagal balance. If sympathetic division dominates persistently for a lengthy duration, it will increase the workload to the heart as the heart is required to pump rapidly and vigorously as long as the fight and flight mechanism is activated which is indicated by a lower HRV. It has been evidenced that the risk to morbidity and mortality are negatively correlated with HRV (Malik *et al.,* 1996; Umetani *et al.,* 1998; Zulfiqar *et al.,* 2009). Clinical utility of HRV is not just confined to the cardiac autonomic evaluation, but likewise employed as a predictive screening tool to assess ANS-related diseases which can be either mentally or physically.

2.5.4 Other Clinical Utilities of Heart Rate Variability

In addition to the prognostic importance in the field of ANS, HRV is clinically related in other medical fields has been proven in many studies. It has been reported that HRV assessment is greatly appreciated for being a prognosis tool to approximate the likelihood of both cardiac and non-cardiac disorders (Kleiger *et al.,* 2005).

The term “myocardial infarction (MI)” is often used in a generic sense as a synonym for heart attack. Carney *et al.* (2001) conducted a finding based on 804 acute MI patients, suggesting that subjects with recent MI have a notable decline of RR variations which in turn, resulted in a higher

vulnerability to mortality. Predomination of sympathetic tone is undesirable. It will tend to reduce the ventricular fibrillation baseline, subsequently lead to ventricular fibrillation and may be susceptible to sudden death (Acharya *et al.*, 2006). Stein *et al.* (2005) carried out a finding with a total of 740 subjects with mean age of 61 ± 10 years to evaluate mortality after myocardial infarction using linear and nonlinear HRV analysis and concluded that mortality increased for patients with post-MI with the evidence of significant depression found in long-term HRV parameters.

HRV analysis provides significant clinical perception into acute autonomic impairment caused by diabetes. Sympathetic tone is activated upon the detection of low blood glucose level (hypoglycaemia) by releasing adrenaline or also known as epinephrine, to prompt the liver to produce glucose, thus increase the blood glucose level. On the contrary, a significant high level of blood sugar level (hyperglycaemia) tends to elicit vagal stimulation and thus induce the release of insulin to restore the homeostasis by depressing the blood sugar level. It has been evidenced that vagal response dysfunction associated with depressed HRV that significantly increased the vulnerability to hyperglycaemia, is a non-invasive marker for diabetes mellitus or even diabetes neuropathy. Kudat *et al.* (2006) conducted linear HRV analysis based on 31 diabetes patients and 30 healthy controls to assess the cardiovascular autonomic neuropathy in diabetic patients. All HRV values plunged significantly in diabetic patients compared with healthy individuals, with the only exception of LF/HF ratio which the significance is relatively imperceptible.

Latest researches described HRV as a powerful prognosis tool which is able to provide comprehension on renal dysfunction. Study of Drawz *et al.* (2013) based on 3245 participants has outlined that patients with chronic kidney disease (CKD) have a higher risk in getting cardiac autonomic neuropathy due to a significant higher level of sympathetic activities with notable low values found in HRV indexes. The outcome has been validated by Oliveira *et al.* (2014), who recruited a total of 32 individuals with CKD at stages 3, 4 and 5, manifesting that patients who suffered from chronic kidney disease have a higher vulnerability to left ventricular hypertrophy which is usually associated with a significant low variability of heart rate. A lower

normalized LF/HF ratio found in patients with stage 5 CKD compare with those in stage 3, indicated that the autonomic modulation deteriorates gradually corresponding to the severity.

HRV analysis has been used to evaluate physiologic status of mental health. Hartmann *et al.* (2019) carried out a finding by recruiting 62 depressive volunteers without antidepressant medication and 65 healthy controls to ascertain the clinical practicality of HRV in assessing mental conditions. Results showed that patients with depression have a considerable low SDNN, rMSSD and pNN50 values compared to healthy individuals. This might be owing to predominance of sympathetic tone in depressive patients as flight and fight mechanism is dominant when a person is under stress. Results have been further supported using frequency-domain method by which, a significant deterioration of vagal tone with the evidence of low HF power in subjects with major depressive disorder (MDD) (Hartmann *et al.,* 2019). Antidepressant treatment has been proved effective to reduce the depression severity, evidenced by a notable improvement in vagal-mediated parameters.

2.6 Factors Influencing Heart Rate Variability

It is important to recognize that there is a variety of mediators that can lead to significant changes on HRV. Indeed, different factors can be considered, depending on their respective degree of significance. These factors include physiological variables such as age, gender, circadian rhythm and genetics; lifestyle factors, for instance, smoking, alcohol abuse, endurance activities and sedentary practice (Sammito and Böckelmann, 2016). Among all of these, the impacts of age and gender deserve attention because of being important or interesting when dealing with the study of HRV (Umetani *et al*., 1998).

2.6.1 Age Influence

It has been evidenced that, the HRV is predominantly affected by age. The relationship between age and HRV has been revealed by many studies regardless either using time-domain or frequency-domain analysis. There is no much controversy in which, the current studies reached to the agreement that most HRV values decreased with increasing age. Since HRV is one of the indicators to examine the state of cardiac system, significant decrease of HRV

may disclosed the fact of increasing incapability of cardiovascular system to adjust itself in responding to the sudden changes (Stein, Kleiger and Rottman, 1997; Bonnemeier *et al.*, 2003; Yukishita *et al.*, 2010; Moodithaya and Avadhany, 2012; Abhishekh *et al.*, 2013).

Negative correlation of HRV parameters with age is supported by Stein, Kleiger and Rottman (1997) and Bonnemeier *et al.* (2003). According to the study of Stein, Kleiger and Rottman (1997), linear analysis based on time-domain HRV parameters was conducted based on 30 younger subjects with mean age of 33 ± 4 years and 30 older individuals with average age of 67 ± 3 years. Notably, aging is normally accompanied by a steady decline of HRV values especially those parasympathetically mediated HRV indexes such as rMSSD and pNN50. These results demonstrated that the autonomic regulation showed a discernable bias towards sympathetic tone with significant degradation of vagal modulation. In the time-domain based HRV study of Bonnemeier *et al.* (2003), overall 24-hour HRV indexes that extracted from 166 healthy volunteers (age 42 ± 15 years), exhibited strong correlation with aging, particularly the SDNNi and rMSSD, with regression powers of -0.64 and -0.62 were noted with aging respectively. A strong negative correlation of rMSSD with aging suggested that the sympathetic activities were dominant over the parasympathetic ones for older people.

A research with 99 healthy subjects (aged 21 to 68) to determine the association between age and HRV was conducted by Yukishita *et al.* (2010), concluded that overall frequency-domain HRV indexes showed negative correlations with age for both sexes using regression analysis, with the only notable exception of logarithmically transformed LF/HF ratio. Men and women showed a high regression power of -0.665 and -0.447 respectively for the logarithmically transformed total power (TP) with increasing of age. Since total power is related with both sympathetic and parasympathetic activities, it is thought to reflect the overall cardiac autonomic regulation. Thus, a significantly higher negative regression power indicated that the autonomic modulation is deteriorated with maturity.

Moodithaya and Avadhany (2012) conducted the research in 267 healthy volunteers between the age group of 6 to 55 years using frequency-domain linear analysis. The results again emphasized on the global reduction

of HRV parameters with healthy aging. Absolute HF power is often considered as standard reference to provide an overview into the state of parasympathetic tone. Thus, it can be concluded that sympathetic activity is predominate in higher age group with the evidence of significant decline of HF power. In addition, study of the age dependence in cardiac autonomic control based on 189 healthy participants with mean age of 33.7 years has been carried out by Abhishekh *et al.* (2013). Parasympathetically-mediated HRV indexes such as HF power, declined significantly for elderly compared to the adolescents and adults. Gradual reduction of HF power further suggested that the vagal tone is nearly diminished with increasing of age, indicating the fact of the dominance of sympathetic tone.

2.6.2 Gender Dependence

Significant changes on HRV are often coupled with gender. Recent studies on the evaluation of autonomic modulation for both male and female provide important insights into the impacts of gender on the alteration of time-domain HRV constants. Actually, it is hard to conclude the influence of gender on HRV due to the results provided by researchers, sometimes conflict. Recent studies were mostly conducted based on time-domain HRV variables and involved both short-term and long-term recordings of heart beats to identify the pattern of sex-dependency on HRV along with growth. Evaluation in terms of time-domain indexes such as SDNN, SDANN, SDNNi, SDSD, sNN50, rMSSD and pNN50, have been carried out to ascertain the relationship between gender and HRV.

Day-night variation has been suggested to be taken into consideration when conducting long-term recordings (Stein, Kleiger and Rottman, 1997; Ramaekers *et al.*, 1998; Umetani *et al.*, 1998; Beckers, Verheyden and Aubert, 2006). The significance of vagal influence during night time has been supported by Stein, Kleiger and Rottman (1997) and Ramaekers *et al.* (1998) with an improvement of time-domain based HRV measures. Beckers, Verheyden and Aubert (2006) showed that parasympathetically mediated constants especially for rMSSD and pNN50, depicted an upward trend for both gender especially during night time. This indicated that vagal tone play a dominant role during night-time, as opposed to what happened in daytime.

Finding was conducted by Silvetti, Drago and Ragonese (2001) to investigate the gender influence of children and adolescents (57 males and 46 females) within the range of 1-20 years old. Results showed that there were no significant gender differences found on SDNNi, rMSSD and pNN50 whereas the SDNN and SDANN in men were markedly higher than in women. SDNN and SDANN increased significantly with maturity and were found to be gender-related. A more gradual improvement of vagal-mediated HRV indexes were observed after 10 years old and were gender-independent.

When stepping into adulthood, all the time-domain HRV parameters differed vastly between male and female with notable higher HRV values of men were observed (Ramaekers *et al.,* 1998; Umetani *et al.,* 1998; Bonnemeier *et al.,* 2003). Recent findings discovered that the overall time-domain measurements were considerably higher for younger men than women. Ramaekers *et al.* (1998) described the effects of gender on HRV by providing the facts which, in 135 women and 141 men aged between 18 and 71 years, all HRV indexes were remarkably lower in women than men. Research conducted by Umetani *et al.* (1998) in 260 healthy individuals (112 male, 148 female aged 10 to 99 years old) again confirmed that, the influence of gender was significant in adults aged below 50 years old. Gender's impacts were demonstrated as such way that none of the HRV indexes were higher in younger women. This is further supported by the study of Bonnemeier *et al.* (2003) according to 81 women and 85 men, with the notable higher rMSSD and SDNNi for men aged 20-29 compared to age-matched women. However, Antelmi *et al.* (2004) provided conflicting result in which, for 653 healthy subjects (292 men and 361 women), overall time-domain HRV parameters were significantly higher among women than men before their fifties, with the only remarkable exception of SDNNi.

Gender dependence on HRV was found to be disappeared approximately at the age of forty and above (Stein, Kleiger and Rottman, 1997; Ramaekers *et al.,* 1998; Bonnemeier *et al.* 2003). Stein, Kleiger and Rottman (1997) reported the significant gender differences were only notable for adolescents, but not for seniors especially in vagal-modulated constants based on the HRV signals extracted from 30 males and 30 females. Ramaekers *et al.,* (1998) likewise explained the gender differences halted by reaching the age of

less than 40, with no significant differences observed in rMSSD and pNN50 for men and women and thus concluded that men and women having comparable parasympathetic modulation. For Umetani *et al.,* (1998), the results again validated the findings by claiming that the gender differences decreased gradually when coming to the age of 30 years old and older, and diminished at their fifties. Study of Bonnemeier *et al.* (2003) upheld the claim, with the evidence of HRV indexes for both men and women converged, demonstrated that the gender-dependency is nearly diminished with increasing age.

Studies on gender influence suggested that women are more cardioprotective than men in the later life. Stein, Kleiger and Rottman (1997) outline that all HRV parameters were higher for younger men in the first instance, followed by a dramatic decline with aging compared to women, suggested a higher level of total autonomic and vagal functions in women in their advanced age. Yukishita *et al.* (2010) carried out a study with 64 males and 35 females, outlining that men demonstrated a remarkable reduction of SDNN values compared to women when moving from twenties towards forties. Since SDNN reflects the total variability of heart rate, the result revealed that the capability and efficiency of cardiac autonomic regulation of men in responding to different demands during their twenties and thirties, but then reversed when approaching the age of forty and above.

Preliminary investigations based on frequency-domain analysis have been carried out by researchers in order to explore the sex-related differences on HRV. In general, frequency-domain HRV measures of men are mostly comparable to that of the women during their adolescent years, suggesting that the cardiac autonomic function is gender-independent for vicenarians. According to Stein, Kleiger and Rottman (1997), all the HRV values of men were found to be similar to women with the only exception of 24-hour, daytime and nighttime LF/HF ratio. A higher LF/HF ratio suggested that men might be sympathetically regulated during their young-age. The results were supported by the Agelink *et al.* (2001), who recruited a total of 173 males and 183 females and reported that there were no significant gender differences found on all the frequency-domain measures for youngsters. Perhaps the only notable difference was the LF/HF ratio in which, the women had the lower

LF/HF ratio compared with age-matched men, indicating that younger women were probably experienced higher level of parasympathetic activities. However, Yukishita *et al.* (2010) objected to the results, demonstrating that the all the frequency-domain HRV indexes were significantly higher in younger men.

Overall HRV values were found to be higher in men against women during their thirties. According to Agelink *et al.* (2001), LF and total power of men were conspicuously higher compared with women except for the HF power, suggesting that there was no gender-related difference in vagal tone during their middle-aged. Yukishita *et al.* (2010) agreed with the finding of Agelink *et al.* (2001), who demonstrated that the gender differences were significant at the age of 30 with the exception of Ln HF power. A notable higher LF/HF ratio was found in men which in turn, indicating that there is a higher comparative sympathetic activity for men compared to women. When stepping into the age of 40, a reversal occurred in which, all the HRV parameters were considerably higher in women than in men, suggesting that sympathetic tone is dominant over vagal tone (Yukishita *et al.*, 2010).

Studies of Stein, Kleiger and Rottman (1997) and Agelink *et al.* (2001) outlined that the gender-related difference diminished with increasing of age. Both studies agreed on the results in which, the sympathovagal function is generally gender-unrelated especially for the age of 50 years old and above. All the HRV values of men were comparable to the women with the only higher HF power was found in women against men, indicating that women are having a comparative high vagal activity compared with men in the later life.

2.6.3 Other Possible Factors

Migliaro *et al.* (2001) has carried out a study to evaluate the degree of association of lifestyle on HRV. In the research, sedentary volunteers were recruited and constituted of both juniors and seniors (9 nonsedentary youngsters, 9 sedentary youngies and 16 sedentary elderly). Sedentary subjects were referred to the individuals who were characterized by much sitting and did very little physical exercise. Vagal-associated parameter, rMSSD was taken into account to ratify the impact of lifestyle on parasympathetic modulation. HRV was expected to decrease with resultant diminution of vagal

modulation for subjects who lead a sedentary lifestyle. However, the result did not showed a significant difference on rMSSD when comparing the sedentary individuals with those non-sedentary subjects of the younger group.

Result of Migliaro *et al.* (2001) has been reviewed by Carter, Banister and Blaber (2003) based on 24 subjects to determine the influence of lifestyle on HRV. All the subjects underwent a 12 week endurance training by performing a 2-mile criterion performance run. HRV indexes were recorded during submaximal exercise upon the completion of endurance training. SDNN increased significantly after endurance training, indicated a remarkable improvement of cardiac autonomic system to meet the demands when coming across different circumstances. This result was further supported by Sandercock, Bromley and Brodie (2005) according to the result of 620 cases, who demonstrated an increment of RR interval with constant physical exercise. Alteration in RR interval suggested a higher level of parasympathetic activity and is ideal to prevent cardiac arrhythmia.

Body mass index (BMI) is believed to be one of the factors that could affect the HRV. Its relevance has been discussed in the finding of by Poirier et al. (2003) who demonstrated an increase of parasympathetic tone and improvement of cardiac autonomic regulation, after a significant decrease in body weight with the evidence of higher HF power recorded in accordance with the result based on 8 severely obese subjects. Influence of body weight is further validated by Vallejo *et al.* (2005). 31 healthy women aged 21 to 35 years old were recruited in this study. Values of SDNN, rMSSD and pNN50 bottomed out for women with BMI less than 19kg/m^2 and greater than 30kg/m^2.

In addition to the significance of BMI on HRV, gonadal hormone shows remarkable effect in varying HRV of women accountable for the disappearance of gender difference in the later life (Liu, Kuo and Yang, 2003; Bonnemeier *et al.,* 2003; Beckers, Verheyden and Aubert, 2006; Yukishita *et al.,* 2010). Effects of estrogen are considerable. When comparing the postmenopausal women with and without estrogen replacement therapy, the latter one possessed a lower high frequency and higher LF/HF fraction, demonstrated that facilitating of estrogen promotes parasympathetic modulation whilst impairs the cardiac sympathetic regulation (Liu, Kuo and

Yang, 2003; Bonnemeier *et al.*, 2003; Beckers, Verheyden and Auber, 2006; Yukishita *et al.*, 2010).

Numerous studies have proposed to take account of disease as one of the factors that could lead to significant changes of HRV. In the research carried out by Cowan *et al.* (1994) based on 206 subjects (95 of them experienced sudden cardiac arrest), the subjects who previously underwent sudden cardiac arrest demonstrated a lower values of time-domain HRV compared with healthy individuals regardless gender, with a more significant differences found in men. Parasympathetically-mediated time-domain based HRV indexes such as rMSSD and pNN50 (%), were found to be slightly higher in women after sudden cardiac arrest. Individuals who suffered from chronic or subacute coronary heart disease, are generally associated with significant lower HRV indexes compared with volunteers who were physically fit (Bigger *et al.*, 1995). In the study of Kallio *et al.* (2002), 32 untreated patients with Parkinson's disease and 24 healthy subjects as controls were included. SDNN and pNN50 were reported lower in subjects who suffered from Parkinson's disease and a more significant reduction was found on pNN50. Apart from the time-domain measures, frequency-domain indexes likewise showed variations in which, absolute total power and normalized HF power were underwent a global reduction whereas normalized LF power and LF/HF ratio increased in patient who suffered Parkinson's disease. Table 2.6 summarised the various possible factors and their corresponding effects on HRV.

Table 2.6: Influence of Various Possible Factors on HRV

Factors	Influence on HRV
Age	Overall HRV parameters were found to be negatively correlated with increasing of age.
Gender	Gender-HRV relations are remained equivocal. SDNN and SDANN are gender-related in which, a higher SDNN and SDANN is observed in men whereas vagal-mediated HRV parameters are gender-independent below 20 years old. When stepping into adulthood, men are conspicuously having higher HRV than age-matched women. Gender-dependence on HRV is found to be diminished about the age of forty and above.
Lifestyle	A notable higher HRV is observed by conducting constant physical exercise such as endurance training.
Body Mass Index (BMI)	Subjects with exceptionally low and high BMI are generally having lower HRV.
Hormone	Hormone such as estrogen promotes parasympathetic activities and thus, a higher HRV is observed in subjects with estrogen facilitation.
Disease	Cardiovascular and non-cardiovascular afflictions such as myocardial infarction and Parkinson disease are perhaps to be one of the factors that will significantly decrease HRV.

CHAPTER 3

METHODOLOGY AND WORK PLAN

3.1 Introduction

This research project is mainly dealt with the statistical analysis of HRV, associates with the possible factors, particularly the age and gender. It is important to recognize the substantive elements in a standard statistical analysis to avoid data misrepresentation. To kick off statistical analysis, the first ever thing that need to be done is development of research questions, followed by setting the hypothesis and significance level. Normality test is then came into play to examine whether the data is normally distributed. If the normality test demonstrates a negative result, data transformation can be called-out to correct the sample distribution to be parametric. Steps that lead to a proper selection of statistical analysis are also discussed under this section.

3.2 Data Description

The data used in this research were the text field data derived from automated analysis of signals which can be acquired and assessed relatively simple. A total of 14399 subjects of which 7275 males and 7124 females between the age of 7 – 100 years old with mean age of 63.80 ± 16.32 and 61.23 ± 18.12 years old were recruited for this research project in a decade's time in which, from year 2008 to year 2018 at Central Cardiology Sydney practice. All participants underwent 24-hour ambulatory ECG monitoring using 3-channel electrograph. ECG recordings were accomplished using Phillips Zymed™ recorders (Phillips, 2020. Amsterdam, Netherlands: Koninklijke Philips N.V.). All the recordings were further interpreted via a proprietary software by an experienced Holter technician and a report was finalized by a competent Cardiologist. Within the report was the noteworthy particulars extracted from the ECG which include pulse rate and variations between adjacent R peaks in succession that saved in test fields. Demographic data such as age and gender and clinical particulars were drawn out using MATLAB 2018b (MATLAB, 2018. version 9.5 (R2018b), Natrick, Massachusetts: The MathWorks Inc). Besides that, natural language processing was employed to identify the

contents of the recordings in which, the exclusively sinus rhythm and occasion of atrial fibrillation were detected.

3.3 Research Questions

It is generally accepted or understood that research questions are worthy of further consideration. Specifying a research question is mandatory and thus, should be implemented whenever a social research is intended to appraise a definite social aspect by looking into the people and societies, in order to accommodate to a wide variety of public demands or requirements. Research question has been established and incorporated to be served as a benchmark within a study to direct the research approaches (Bryman, 2007). In general, research question initiates a research project and frequently used as the first operative step. In this study, three research questions have been outlined based on interests of the study:

a) Does gender affect heart rate variability?
b) Does age affect heart rate variability?
c) Does the influence of gender on heart rate variability diminishes with increasing of age?

3.4 Statistical Hypothesis Testing

Statistical hypothesis testing is recognized as one of the most ideal assessment in which, decision making can be done effortlessly. It is performed to declare about the nature population and commonly expressed in terms of population parameter (Ross, 2017). To kick-start statistical hypothesis testing, it is important for researcher to recognize the essential elements which are null and alternative hypothesis. Null hypothesis, which is denoted by H_0, is came into existence when the researcher holds the opinion that an alteration of independent variable does not give rise to any variation in dependent variable. There is no relation between two variables if null hypothesis is being accepted. On the contrary, alternative hypothesis, as the name implies, is an attempt that refute what null hypothesis declared. It is generally denoted by H_1. If alternative hypothesis is being acknowledged, there is a significant difference

or impact on the dependent variable whenever the independent variable is being modified.

3.4.1 Null and Alternative Hypothesis

Null and alternative hypothesis have been developed according to the research questions, referring to the statements stated below:

For research question (a):

H_o: Male and female have similar HRV.

H_1: Male and female have different HRV.

For research question (b):

H_o: There is no significant change on HRV with increasing of age.

H_1: There is significant change on HRV with increasing of age.

For research question (c):

H_o: Gender does not influence HRV with increasing of age.

H_1: The influence of gender on HRV changes with increasing of age.

3.4.2 Probability Value (p-value)

Probability value, which is often abbreviated as p-value, is the substantial part of statistical hypothesis testing. It is represented within the range of 0 to 1 and used to specify the smallest significance level to promote a knock-back of null hypothesis. If p-value calculated is much smaller than standard significance level, indicated that the researcher has a stronger proof to accept alternative hypothesis. In general, there are two significance levels that frequently implemented within a statistical hypothesis research which are the 5% and 0.1% levels of significance. Significance level of 0.05 serves as a cut-point in which, if a computed p-value is smaller than 0.05, null hypothesis rejection is inevitable and the results are considered to be statistically significant whilst null hypothesis is failed to reject if a calculated p-value is greater than that of the 5% level of significance. It same goes to the 0.1% level of significance, with the only exception of the degree of significance. If the probability value calculated is smaller than 0.001 ($p < 0.001$), demonstrated that the results are clinically significant.

3.5 Normality Test

Normality test is the assessment that particularly applied in the statistical analysis to unmask the likelihood for an arbitrary variable underlying the data set whether it is normally distributed. It is widely adopted to evaluate whether the data set is perfectly resembled a normal distribution. To date, there are various methods to examine the data normality. Of these, three stand out: graphical method, frequentist test, skewness and kurtosis analysis.

3.5.1 Graphical Method

Sample data can be illustrated in a graphical representation by firstly computing the random data into a histogram. The histogram of the data set is then further compared to a normal distribution curve, which is likewise known as bell curve. Sample data is recognized as well-normalized if it is demonstrated as a bell-shaped and bore a resemblance to normal distribution. Normal distribution is characterized by few properties in which, the mean, mode and median are equivalent to each other; a symmetrical curve is observed which is located at the middle of the histogram; and lastly, the total area under the curve must be a value of 1. Span of the distribution is predominantly directed by the standard deviation. A narrower and taller normal distribution is observed for a smaller standard deviation, suggested that the sample data is closely congregated around the mean. On the contrary, a greater standard deviation outstretched the distribution width, results in a flatter and wider normal distribution which in turn, further outlined that the data is outspreaded throughout the mean. Empirical rule which is also known as three-sigma or 68-99.5-97 rule, is applied to regulate the proportion of value that falls within a certain number of standard deviations (σ) from the mean (μ), generally expressed in percentage (%). Figure 3.1 depicted a normal distribution curve that is corresponded to empirical rule (McLeod, 2019).

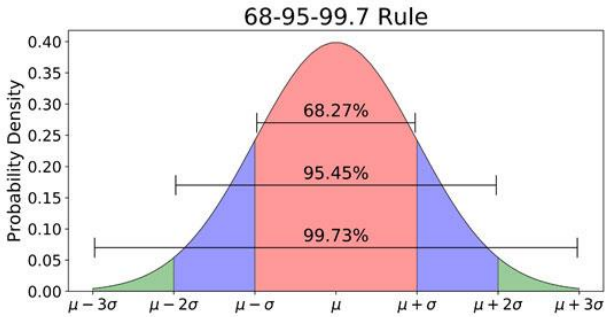


Figure 3.1: Normal Distribution Curve

Normality of the sample data can be evaluated using normal probability plot and quantile-quantile plot aside from the conventional bell-shaped curve. Elements that lead to a significant deviation from normality can be determined via normal probability plot. These components include outliers, skewness, kurtosis and the necessity for data transformation. A linear line is obtained if the data set is perfectly modelled by a normal distribution. Points that clustered around the line indicated the sample data which is well-fitted a normal distribution. Data items that are lack of normality can be easily detected in which, they are conspicuously out of line with rest of the data. Figure 3.2 illustrated a normal probability plot (Karen, 2020). The data are normally distributed as a straight line is exhibited with almost all the points bunched up around the line. On the other hand, quantile-quantile plot, as abbreviated as Q-Q plot, is one of the graphical statistical analysis that assess normality by comparing quantiles of the data set against the quantiles from normal distribution to identify whether if two sets of data are derived from same distribution. If the points are well-fitted a straight 45º line, suggested that the two sets of data are of the identical distribution. Figure 3.3 (Statstutor, 2020) showed sample data plotted using Q-Q plot. The points follow a strongly nonlinear pattern, indicating the data are not normally distributed as the points are mostly deviated away from the 45º line.

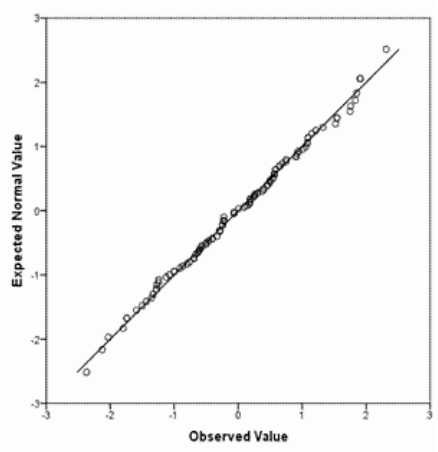


Figure 3.2: Normal Probability Plot

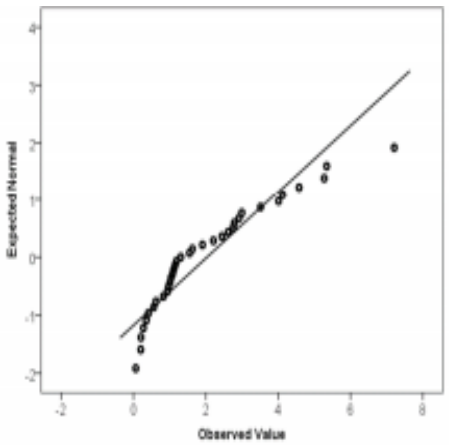


Figure 3.3: Q-Q Plot

3.5.2 Frequentist Test

Frequentist test is recognized as one of the most frequently used assessment tools in determining whether the sample data is normally distributed. However, its utility has been disputed in which, the frequentist testing is not suited to be employed within the analysis with large sample data (Oraguzie *et al.*, 2007). Data are examined by applying statistical hypothesis testing in which, null hypothesis is agreed with the statement that data is well-modelled by a normal distribution. There are many frequentist tests available currently and perhaps the most practicable are the Anderson-Darling, Kolmogorov-Smirnov and Shapiro-Wilk test. This is further supported by Razali and Yap (2011) in the study, demonstrated that the Shapiro-Wilk has the greatest statistical power, followed by the Anderson-Darling and lastly the Kolmogorov-Smirnov test.

Shapiro-Wilk test is well-known to be the most powerful normality tool that identifies the distribution of data variable. Samples are randomly selected and plotted into a histogram. The histogram is then further being compared to the normal distribution curve. Normal distribution curve is laid on top of the histogram, enabling the researcher to figure out the resemblance between them. Similarity percentage is then calculated based on the overlaps of sample data between the examined data and normal distribution. The probability of obtaining this similarity percentage is further sorted out. Statistical hypothesis testing came into place as the final step in which, null hypothesis is accepted if probability computed is greater than 5% level of significance, suggested that the data are normally distributed.

Kolmogorov-Smirnov (K-S) test has the self-same intention as the Shapiro-Wilk test, with the notable exception of performance in which, the former has lower statistical power than the latter one. There are two K-S tests in which, the one-sample case is dealt with the comparison between sample and that of a reference probability and two-sample K-S test which is applied when comparison is performed using two samples. In the K-S test, null hypothesis is accepted when the probability distribution computed is greater than that of the significance of 0.05 which in turn, suggests that the samples are drawn from the same distribution. The statistical utility of K-S test is often limited by its extremely high sensitivity to extreme values (Ghasemi and Zahediasl, 2012). Anderson's Darling test has been proven to be competent for goodness of fit test which is performed to examine the fitness of a variable to a particular distribution. When the computed probability value is smaller than 0.05, null hypothesis which is stated that the data is derived from a particular distribution, is strongly rejected.

3.5.3 Skewness and Kurtosis

Skewness is a non-invasive marker to evaluate the symmetry of the probability distribution. Data set is said to be symmetrically distributed if the distribution is made up of exactly similar parts facing each other or around an axis. If the sample data is not well-modelled by a normal distribution curve, it can either be positively skewed in which, the distribution has a right-skewed curve or be negatively skewed in which, a left-skewed curve is observed. Besides of the conventional positive and negative skew, probability distribution of a random selected variable can likewise to have zero or undefined skew. Left-skewed distribution, as the name suggests, is characterized by a notable flatter tail on the left side whilst a conspicuous longer tail is observed on the right side of the distribution, referred as right-skewed.

Skewness is indirectly correlated with mean and median. A normal distribution is always assumed that the mean, mode and median are equal to each other. However, a negatively skewed distribution has a greater median than mean whereas for a positively skewed distribution, mean is found to be greater than median. Skewness can be computed using two methods which are the Pearson's first coefficients of skewness (mode skewness) and Pearson's

second coefficients of skewness (median skewness). Skewness of (-1,1) is an acceptable range for a distribution being normally distributed in accordance with a rule of thumb in which, a highly skewed distribution has a skewness that less than -1 or greater than 1 (Medium, 2020). However, there is disagreement saying that, the distribution is considered as normally distributed if the skewness is in between -1.96 and 1.96 (Kim, 2013). Figure 3.4 (Statistics How To, 2020) demonstrated distributions with negative (left side) and positive (right side) skewness.

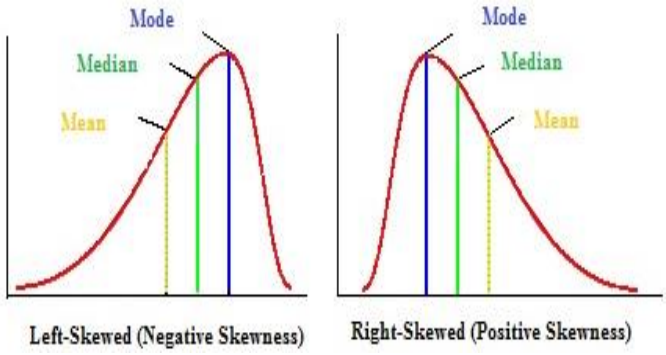


Figure 3.4: Distributions with Negative and Positive Skewness

Kurtosis is always applied along with skewness to evaluate the distribution. Kurtosis measures the sharpness of the peak of a probability distribution by taking into account both tails of the distribution. Greater kurtosis is obtained when the tailed data of the distribution is surpassed that of the normal distribution, results in a heavier tails. Data set is said to be normally distributed if the kurtosis is within the range of (-3,3) (Kallner, 2018). There are three types of kurtosis that used to indicate the “tailedness” of a distribution, include mesokurtic, leptokurtic and platykurtic. Mesokurtic distribution is somehow comparable to the normal distribution in which, similar kurtosis is observed. Distribution with kurtosis greater than 3 is likewise known as leptokurtic distribution in which, a heavier tails associated with an increase of outliers, is demonstrated when compared to a normal distribution. Platykurtic distribution has a kurtosis that is less than 3. This type of distribution will result in a lighter tails with a significant fewer extreme outliers than that of normal distribution. Figure 3.5 depicted the three types of kurtosis (AnalystPrep | CFA Study Notes., 2020).

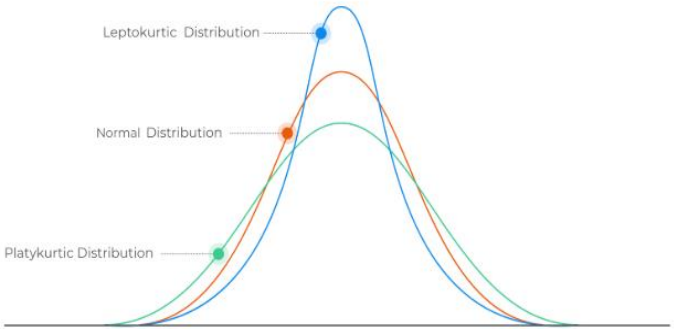


Figure 3.5: Mesokurtic, Leptokurtic and Platykurtic Distributions

3.5.4 Summary

Since all the tests evaluate normality in their particular way, a summarized table might provide an overview on how the tests are performed to achieve their ultimate intention, referring to the Table 3.1.

Table 3.1: Summary for Normality Tests

Normality Test	Method	Explanation
Graphical	Normal distribution curve (normally plotted as histogram)	If the distribution is resembled a bell-shaped, it is said to be normally distributed in accordance with the following assumptions which are: mean must be equal to mode and median; a symmetrical curve is observed and total area under the curve equivalent to 1.
	Normal probability plot	Linear line is obtained if the samples are well-modelled by a normal distribution.
	Quantile-quantile plot (Q-Q plot)	Quantiles of two probability distributions are used to assess whether they are derived from same distribution using a 45^{o} line.
Frequentist	• D'Agostino's K-squared test • Jarque-Bera test • Anderson-Darling test • Cramér-von Mises criterion • Kolmogorov-Smirnov test • Lilliefors test • Shapiro-Wilk test	Of these, three stand out: Anderson-Darling test, Kolmogorov-Smirnov test and Shapiro-Wilk test. Statistical power is arranged in such way: Shapiro-Wilk test > Anderson-Darling test > Kolmogorov-Smirnov test. Hypothesis testing is applied in which, data are examined against the null hypothesis which is claimed that the data is followed a normal distribution.
Skewness	Negative skewness	Distribution is left-skewed with longer tail at the left side. Greater median value is found compared to mean.
	Positive skewness	Distribution is right-skewed with longer tail at the right side. Greater mean value is observed compared to mean.
Kurtosis	Mesokurtic	Has kurtosis statistic similar to that of the normal distribution
	Leptokurtic	Has kurtosis that greater than 3 and heavier tails with more outliers than the normal distribution
	Platykurtic	Has kurtosis that less than 3 and lighter tails with fewer outliers than the normal distribution

3.6 Data Transformation

Data transformation is came into place when the data is failed to be distributed normally which is validated by the normality test. It is a process in which, a positively skewed or negatively skewed data is converted to a data with centred distribution. There are few alternatives provided to transform the data, but it is important to identify the skewness of the data and perhaps, the values of the variable as data transformation is mostly applicable to the positive and non-zero values.

To date, there are roughly six types of data transformation available to correct the data skewness. Positively skewed data can be transformed via logarithmic, square root and reciprocal transformation. On the other hand, exponential and power transformations are particularly designated for negatively skewed data. Of these, logarithmic transformation is further intended for data that might have exponential element and cumulative effects which are subjected to or of the nature of multiplication. Skewed data can be transformed using base-2, base-10 or base-e logarithmic function.

Square root transformation is intended for data that may be presented and expressed as counts or frequencies; data that consists many zero or exceptionally small values and data that might have a physical element whereas data that can be expressed as a quotient can be transformed using reciprocal transformation. On the contrary, exponential transformation is ideal for left-skewed data that may have logarithmic pattern. Power transformation is likewise applied to reduce the negative skewness for data that might have a physical element. Lastly, data that are represented as a proportion or percentage in which, data points are mostly fell between 20 percent and 80 percent can be well-transformed using arcsine transformation.

3.7 Procedures of Statistical Analysis

In this research project, 24-hour time-domain HRV indexes were computed. These include SDNN, SDANN, SDNN index and rMSSD which were being selected in accordance with the Task Force Report (Malik *et al.*, 1996). 5% and 0.1% levels of significance were used to examine whether the result is generally and clinically significant respectively. Null hypothesis was rejected at $p < 0.05$.

Frequentist tests have been proven to be ineffective to work with large sample size (Oraguzie et al., 2007). On the other hand, the usability of skewness and kurtosis have likewise been disputed in which, the acceptable range aroused a controversy among the researches (Kim, 2013; Kallner, 2018; Medium, 2020). Perhaps the simplest and easiest to perform was the graphical method when frequentist test might be over- or under-sensitive (Gupta *et al.*, 2019). The standard normal curve is most appropriate owing to its greater statistical power to evaluate large sample size (Heiman, 2014). Therefore, data normality were determined by graphical method include both normal distribution curve and normal Q-Q plot. Base-10 logarithmic function was implemented to correct the distribution skewness.

Statistical analysis was conducted using Statistical Package of Social Sciences (SPSS) software (IBM Corp. Released 2018. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). A proper choice of statistical analysis is crucial to disclose the underlying relations between two subjects of interest. Thus, a flowchart summarizes the essential components that should be taken into consideration which in turn, leading to the selection of an appropriate approach to examine the stated hypothesis, has been established corresponding to Figure 3.6 (Gerwien, 2020).

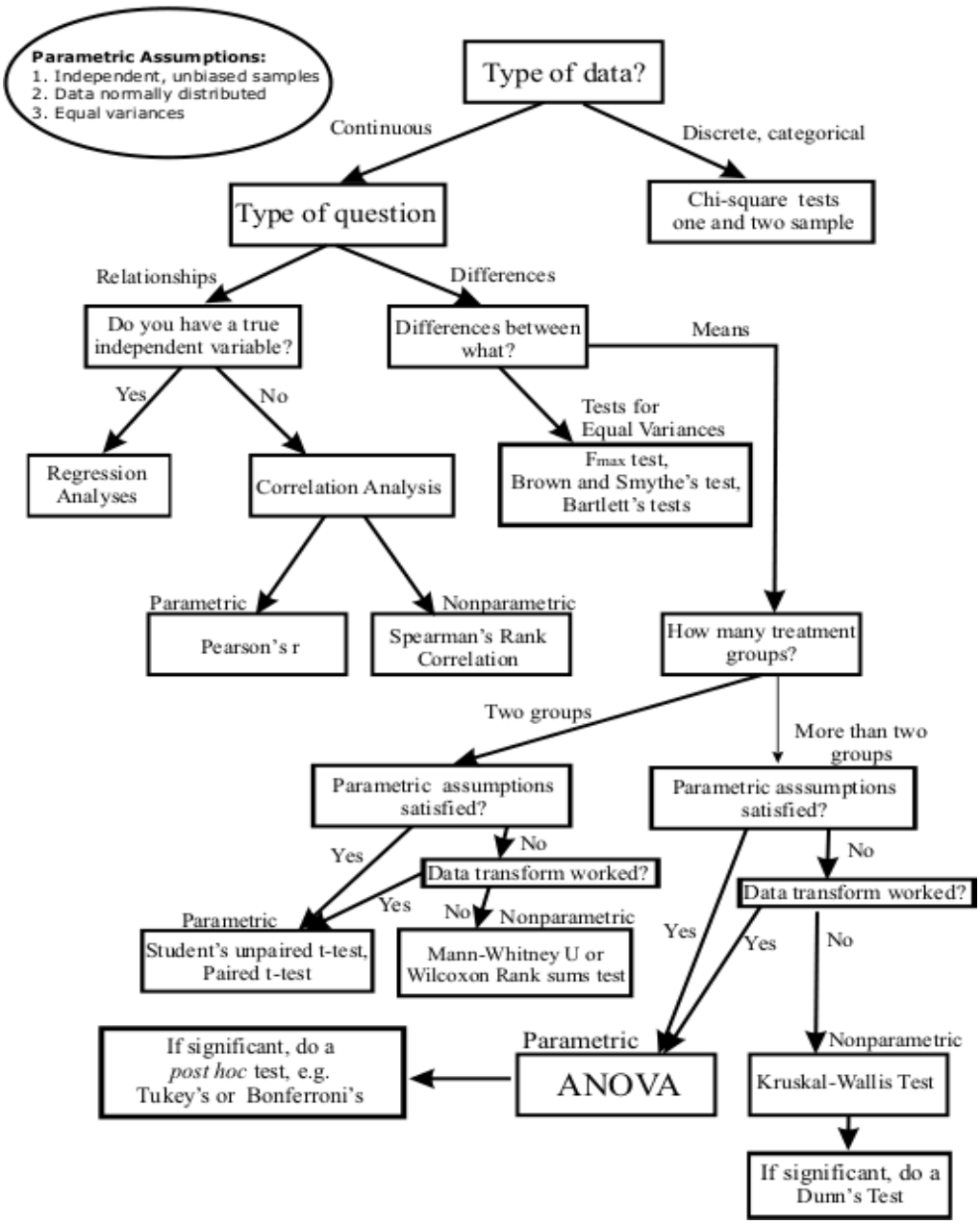


Figure 3.6: Flowchart for Selecting Commonly Used Statistical Tests

3.7.1 Statistical Test 1

Test 1 was corresponded to the research question (a) in which, the impact of gender on heart rate variability was investigated. Since variations between R peaks in succession were continuously monitored and traced without interference, it was considered as a continuous data rather than being a discrete random data. Thus, chi-square test that is used for categorical data, was not considered to be implemented. It was required to consider the lower and upper 95% confidence limits of 24-hour HRV as suggested in the study of Umetani *et al.* (1998) to define the normal HRV range. According to Umetani *et al.*

(1998), HRV values should be within the range of which, SDNN (53 – 279 ms); SDANN (45 – 261 ms); SDNN-i (0 – 113 ms); and rMSSD (7 – 103 ms), to be considered as normal. Any cases having the values fell outside the normal range were considered as invalid cases and should be filtered out.

To determine the relationship between the gender and HRV, it was necessary to take into account the regression and correlation analysis. Since the variables were designated neither as dependent nor independent, thus correlation analysis was selected. Pearson's r correlation coefficient and Spearman's rank correlation coefficient are the most commonly used for correlation test in which, the former is ideal for quantitative data whilst the latter is recommended for qualitative data. The term 'parametric' is widely adopted within statistical analysis to indicate that the data are normally distributed. Parametric tests are intended for continuous variables and quantitative data which are either based on interval or ratio scales of measurement whilst the nonparametric tests are primarily designated for qualitative data such as ordinal and nominal data which are generally not normally distributed. To perform parametric test, the data must attained the requirements in which, the data are normally distributed, leastways symmetrically allocated; data collected from different subject groups, have the equal variance; and the data are gathered from independent and impartial samples. Since 24-hour time-domain HRV parameters were computed based on the interval between successive R peaks, parametric Pearson's r correlation coefficient was employed to quantify the association with gender. Two-tailed Pearson's correlation was performed since the relationship is neither a positive nor negative.

HRV parameters were initially followed a normal distribution with the only exception of rMSSD which was evidenced by the normality test using graphical method. Logarithmic data transformation in base 10 was then applied to correct the skewness. Data were successfully modelled by a normal distribution after data transformation. Either mean or median can be utilized to measure the differences between subjects groups in which, differences of parametric data can be evaluated using means while the medians can be used to evaluate the differences of nonparametric data. For this research question, means were being the interest of study as the data were parametric.

Means can be exploited by firstly looking into the number of subject groups. Subjects were split into two groups according to their gender to study the effect on HRV respectively. Thus, student's t-test was implemented. Student's t-test is further classified into two types which are the unpaired t-test and paired t-test. Unpaired t-test is also known as independent t-test which is utilized when sample data are derived from separate individuals whereas paired t-test, which is also called as dependent t-test, is intended when the data arise from the same individual with different points in time. Student's unpaired t-test was thus being selected as the data were collected from different subjects to evaluate the gender difference on HRV. Figure 3.7 illustrated the flowchart which summarized the steps that led to the final decision on the statistical test used.

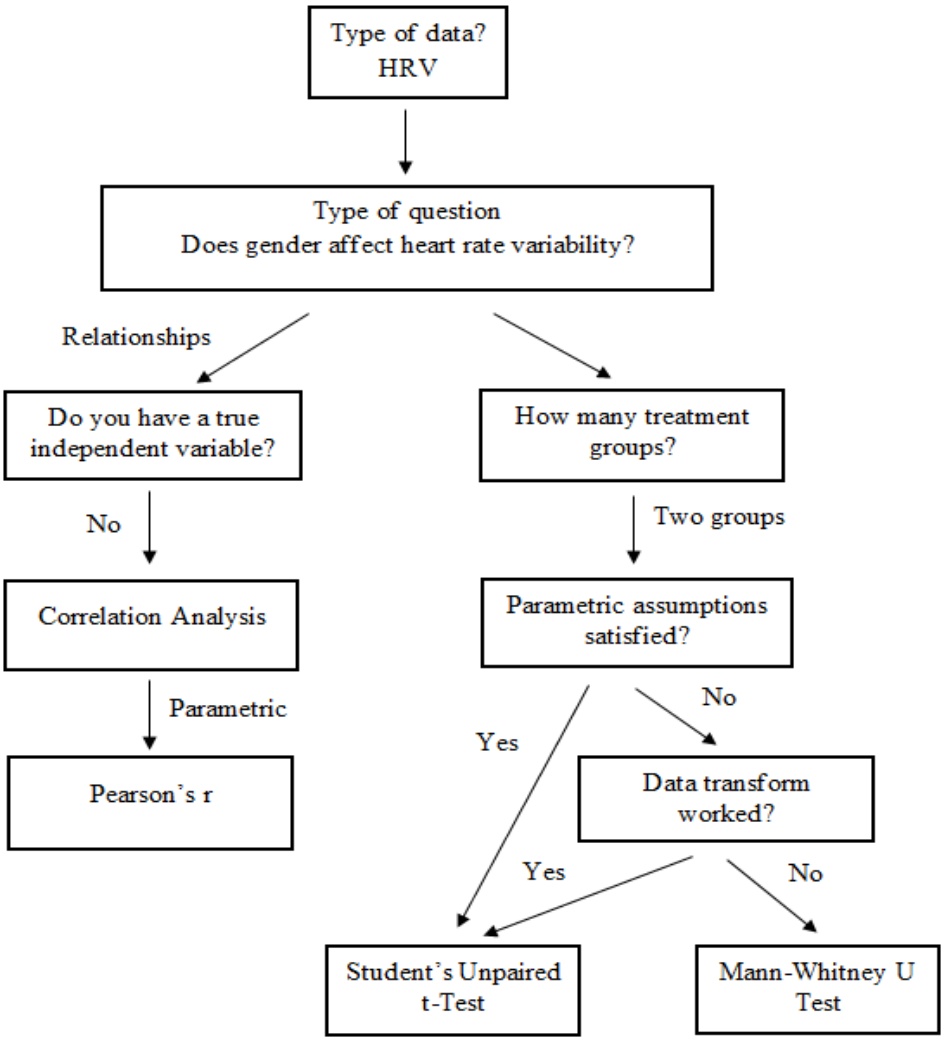


Figure 3.7: Flowchart for Statistical Test 1

3.7.2 Statistical Test 2

For statistical analysis 2, it was referred to the research question (b) that was intended to reveal the significance of age on HRV. Time-domain HRV indexes were acquired from the variations of heart rate which were continuously monitored using ECG. Therefore, it is classified as continuous variable rather than categorical as the data formed in sequence. Invalid cases were eliminated by following the setting done in statistical test 1. Relationship between the age and HRV was discovered using correlation analysis since a true independent variable was absent in the statistical study for research question (b). Parametric Pearson's r correlation analysis was incorporated to measure the degree of association between the age and HRV as the HRV indexes have been proved to be well-shaped by a normal distribution after performing the normality test and data transformation. Two-tailed Pearson's correlation was chosen to test the significance as the degree of association is not measured either positively or negatively. Correlation of HRV with maturity was first observed without age group classification.

Participants were then divided into eight decades based on their age as illustrated in Table 3.2. HRV computed were observed as being normally distributed. However, rMSSD was illustrated as a positively skewed curve under graphical method of normality test. Fortunately, logarithmic base 10 data transformation worked well to normalize the distribution. Differences between each age group were determined using means as the data were well-modelled by normal distribution. Since there were eight groups of subjects, analysis of variance (ANOVA) test was performed to assess the equality of means between two or more independent groups. In general, there are two ANOVA tests available which are the one-way ANOVA and two-way ANOVA in which, the former option is intended for statistical analysis that consists of only one independent variable whilst the latter one is applied when there are two independent variables. One-way ANOVA test was thus selected to determine the impact of age which is the only one independent variable, on HRV.

One-way ANOVA test is capable to unmask the differences between groups. However, it would not be able to reveal the condition in which, which groups were different. If the result yielded a statistical significance, a post hoc

test is required to confirm that which groups had a difference in means. There are many post hoc tests available to analyse the result based on the interest of study. Tukey's post hoc test was picked out as being the assessment tool to sort out which groups in the sample differ. This was done by compare every mean with every other mean using Honest Significant Difference. All steps that eventually lead the way to a proper statistical analysis were summarized in the flowchart as depicted in Figure 3.8.

Table 3.2: Age Group

Group	**Age**
1	< 20
2	20 – 29
3	30 – 39
4	40 – 49
5	50 – 59
6	60 – 69
7	70 – 79
8	≥ 80

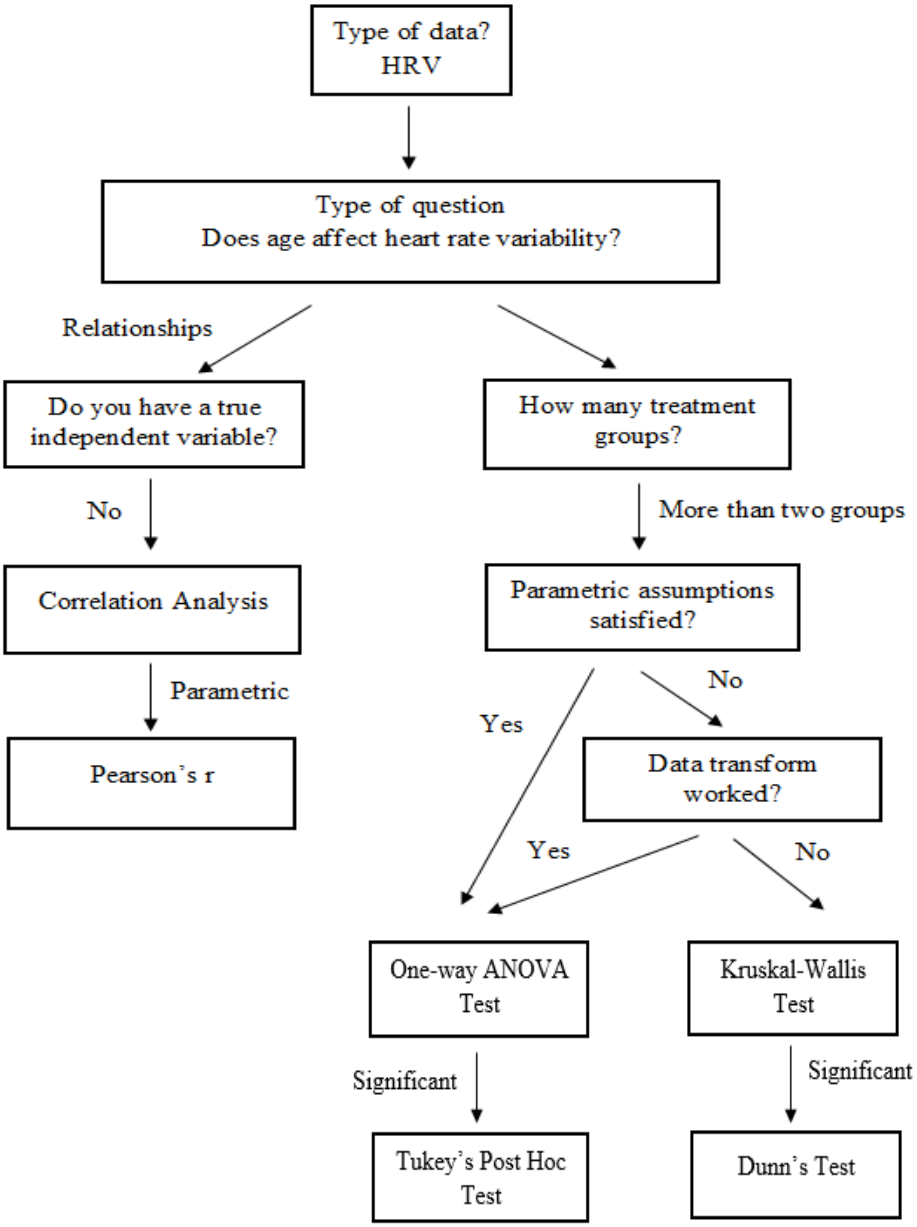


Figure 3.8: Flowchart for Statistical Test 2

3.7.3 Statistical Test 3

Test 3 was concerned about the research question (c) which the gender dependence with increasing of age was evaluated. The data were obtained based on the continuous ECG recording. Therefore, a discrete chi-square test was not employed. Valid cases were selected in accordance with the setting employed in the statistical analysis 1. To study the gender difference with maturity on HRV, regression analysis was preferred to correlation analysis as there was at least an exact independent variable. Independent variable is referred as a variable whose variation does not depend on that of another

whereas the dependent variable is indicated as a variable whose value depends on that of another. Regression analysis was used when the interest of study was focused on the prediction of a continuous dependent variable, in this case was the HRV, from a number of independent variables which were the age and gender. There are a wide variety of regression analysis available, take linear regression, logistic regression and ridge regression as examples. Of these, multiple linear regression analysis that under the category of linear regression was chosen as there are two independent variables used to predict the value of a dependent variable.

Volunteers which have been separated in accordance with age, were further split into two groups based on their sexes. Normality test was conducted prior to the statistical test. All the HRV parameters were normally distributed except for rMSSD which was illustrated as a right-skewed curve and thus, the log 10 data transformation was came into play. Since the data were parametric after logarithmic transformation, means were used to assess the differences between groups. Two-way ANOVA test was performed to assess the influence of two nominal predictor variables (age and gender) on a continuous outcome variable (HRV). Tukey's post hoc test was carried out to find out exactly where those differences lied. Figure 3.9 outlined the flowchart that included all the initiatives which led to the correct choice of statistical analysis.

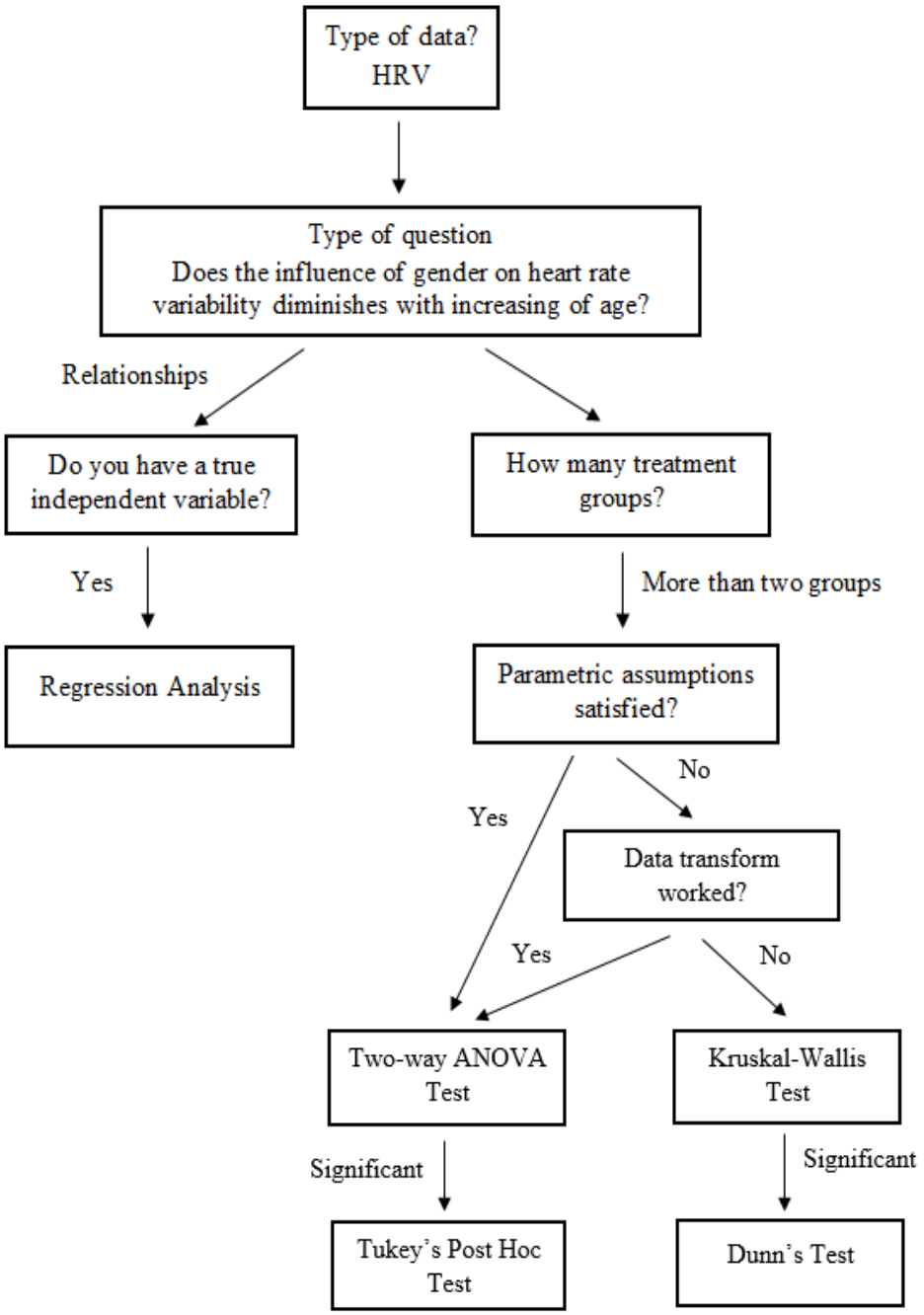


Figure 3.9: Flowchart for Statistical Test 3

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Demographics Data Analysis

The results of statistical analysis for 24-hour time-domain HRV indexes will be discussed in chapter 4. Outcome of data normality and the respective action to correct the data skewness will be likewise depicted under section 4.2. Section 4.3 further assessed the gender influences, aging effects and gender dependence with increasing of age on HRV.

A total of 14399 patients who undergo 24-hour ambulatory ECG monitoring within age of 62.53 ± 17.28 years old were recruited in this study. Of these, 7275 males and 7124 females with a mean age of 63.80 ± 16.32 years old and 61.23 ± 18.12 years old respectively, were involved. A total of 3754 invalid cases (26.07%) were filtered out by eliminating the cases which fell outside the range of normal values for each time-domain HRV parameters. Among 14399 subjects, 10645 of them (73.93%) with mean age of 59.82 ± 17.35 years old which include 5006 males (61.03 ± 16.67 years old) and 5639 females (58.75 ± 17.86 years) that amounted to 47.03% and 52.97% of the total valid subjects respectively, were remained in the study. Table 4.1 presents the age distribution of subjects by decade, while Table 4.2 depicts the heart rate profile of the subjects.

Table 4.1: Age Distribution of Subjects by Decade

Group	Age (years)	Number of Subjects
1	< 20	144
2	20 – 29	627
3	30 – 39	844
4	40 – 49	1163
5	50 – 59	1734
6	60 – 69	2565
7	70 – 79	2360
8	≥ 80	1208

Table 4.2: Heart Rate Profile of Valid Subjects

Heart Rate Profile	**Mean ± s.d.**			**P-value**
	Total	**Gender**		
	(n = 10645)	**Male**	**Female**	
		(n = 5006)	**(n = 5639)**	
Age (years)	59.82 ± 17.35	61.03 ± 16.67	58.75 ± 17.86	< 0.001**
MinHR (beats/min)	49.66 ± 8.04	48.31 ± 8.04	50.86 ± 7.84	< 0.001**
AvgHR (beats/min)	73.19 ± 10.66	71.27 ± 10.75	74.89 ± 10.29	< 0.001**
MaxHR (beats/min)	123.33 ± 22.02	119.60 ± 21.98	126.63 ± 21.51	< 0.001**

** $p < 0.001$; s.d. = Standard Deviation; MinHR = minimum heart rate; AvgHR = average heart rate; MaxHR = maximum heart rate.

4.2 Data Normality

All the HRV profile data were plotted into histograms and the histograms were used to compare with a normal distribution curve. Overall HRV values were said to be approximately normally distributed, with little positive skewness was observed for SDNN, SDANN and SDNN-i. The histograms plotted were strongly resembled a bell-shaped which a normal distribution curve bore with. The only notable exception was found on the rMSSD in which, the distribution was conspicuously being right-skewed.

The normality of data was again validated using normal Q-Q plot. In general, SDNN, SDANN and SDNN-i were more or less being modelled by a normal distribution. High linearity was observed in which, the points were mostly followed a linear pattern and clustered around the straight line. On the contrary, normal Q-Q plot of parameter rMSSD indicated that the data were not normally distributed. This was evidenced by the non-linearity in which, most of the points deviated from the straight line.

Logarithmic transformation was implemented to correct the skewness of rMSSD data. Of these, base 10 logarithmic function was found to be the most effective and thus being used. Data transformation worked well, with the evidence of a higher resemblance to a normal distribution curve was observed. In addition, log 10 transformation has been proved to be effective, proved by a notable improvement of linearity in which, points were mostly clumped around the straight line.

4.3 Statistical Analysis

This section of the research discussed about the outcomes of statistical analysis according to three research questions that have been established in the earlier stage of research project. The results obtained from each statistical analysis will be further evaluated by comparing and contrasting them with the published studies.

4.3.1 Influences of Gender

Table 4.3 depicts the results of two-tailed Pearson's r correlation test to measure the associations between gender and the 24-hour time-domain HRV indexes. Correlations between gender and 24-hour time-domain HRV indexes were said to be clinically significant ($p < 0.001$), outlining that the overall HRV indexes exhibited positively weak correlations with gender in which, correlation coefficients of 0.071, 0.054, 0.104 and 0.077 were reported for SDNN, SDANN, SDNN-i and rMSSD respectively.

Table 4.4 manifests the gender effects on 24-hour time-domain HRV indexes. Gender differences were found to be clinically significant ($p < 0.001$) for all the HRV indexes, suggesting that male and female have different HRV. Overall HRV variables were found to be notably lower in female subjects compared to that of male subjects with reference to Table 4.4. Table 4.5 shows the correlations between HRV parameters, suggesting that all HRV measures were statistically correlated with each other ($p < 0.001$). Using SDNN measure, SDANN, SDNN-i and rMSSD exhibited a strong ($r = 0.972$), moderate ($r = 0.716$) and weak ($r = 0.424$) correlation respectively. For SDANN, SDNN-i has been found to be moderately correlated ($r = 0.580$) while rMSSD was weakly correlated as reported by a significant lower correlation coefficient of 0.306. HRV determined by SDNN-i was moderately correlated with rMSSD with a correlation coefficient of 0.686 was obtained.

Table 4.3: Correlation Between Gender and 24-hour HRV Parameters

Time-domain HRV index	**Correlation Coefficient (r)**
SDNN (ms)	0.071**
SDANN (ms)	0.054**
SDNN-i (ms)	0.104**
rMSSD (ms)	0.077**

** $p < 0.001$; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-min duration; rMSSD = root-mean-square of the successive NN intervals difference.

Table 4.4: Gender Effects on 24-hour Time-domain HRV Parameters

Time-domain HRV Index	**Mean ± s.d.**		**P-value**
	Male (n = 5006)	**Female (n = 5639)**	
SDNN (ms)	135.20 ± 41.19	129.63 ± 36.62	< 0.001**
SDANN (ms)	119.76 ± 39.17	115.77 ± 35.18	< 0.001**
SDNN-i (ms)	54.81 ± 18.45	51.13 ± 16.78	< 0.001**
rMSSD (ms)	41.22 ± 20.68	38.39 ± 20.14	< 0.001**

** $p < 0.001$; s.d. = Standard Deviation; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-min duration; rMSSD = root-mean-square of the successive NN intervals difference.

Table 4.5: Correlation Between 24-hour Time-domain HRV Parameters

	SDNN		**SDANN**		**SDNN-i**		**rMSSD**	
	r	p-value	r	p-value	r	p-value	r	p-value
SDNN	-	-	0.972	< 0.001**	0.716	< 0.001**	0.424	< 0.001**
SDANN	0.972	< 0.001**	-	-	0.580	< 0.001**	0.306	< 0.001**
SDNN-i	0.716	< 0.001**	0.580	< 0.001**	-	-	0.686	< 0.001**
rMSSD	0.424	< 0.001**	0.306	< 0.001**	0.686	< 0.001**	-	-

** $p < 0.001$; r = correlation coefficient; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-min duration; rMSSD = root-mean-square of the successive NN intervals difference.

Previous researchers have outlined that HRV differed by gender in 24-hour HRV, with values for female subjects being significantly lower than those for male subjects. In this regard, Ramaekers *et al.* (1998) concluded that overall HRV indexes were reported higher in male subjects with the utilisation of SDNN, SDANN, rMSSD and pNN50 based on 276 healthy subjects with 135 women and 141 men between 18 and 71 years old. Among the HRV parameters measured, gender differences were only found to be significant for SDNN ($p = 0.049$) and SDANN ($p = 0.047$), but not for HRV parameters denoting vagal modulation such as rMSSD and pNN50 ($p > 0.05$), suggesting that men and female have similar vagal tone.

Apart from the study of Ramaekers *et al.* (1998), the results from statistical analysis are relatively consistent with the findings of Bonnemeier *et al.* (2003). In the research, 24-hour time-domain HRV analysis in 81 healthy women and 85 physically fit men, ranged between 20 to 70 years with mean age of 42 ± 15 years was conducted to evaluate the effects of gender on cardiac autonomic nervous modulation in terms of SDNN, SDANN, SDNN-i, rMSSD and pNN50. In general, all the HRV indexes were found to be gender-related and significantly lower in women compared to men ($p < 0.01$) with the notable exception of vagal-mediated parameters in which, rMSSD and pNN50 were gender-independent, indicating that vagal modulation in men was comparable to that in women.

It is noted that the results obtained from the analysis are somehow being consistent with those of Ramaekers *et al.* (1998) and Bonnemeier *et al.* (2003) except for the findings regarding to the gender difference for rMSSD. Although the results conflicted the outcomes of the existing publications, suggesting that there was gender difference presented for rMSSD in which, rMSSD was significantly higher in men, it is believed that it was the result of having different amount of subjects which is approximately several-fold compared to those existing researches, and perhaps the age range of subjects targeted in the study.

Variations in heart rate determined by SDNN, is known to be highly-correlated with total power which makes this measure capable to reflect both sympathetic and parasympathetic activity accountable for global autonomic regulation of heart rate. Therefore, the significant gender-related difference

found in SDNN in this study suggested that cardiac autonomic regulation is substantially higher in men compared to women with the evidence of scoring a conspicuous higher SDNN value in male subjects. Time-domain SDANN established a strong correlation with SDNN ($r = 0.972$, $p < 0.001$), again validated the results which proved that autonomic modulation of heart rate in men is remarkably higher than women owing to a higher SDANN value reported.

Parameter SDNN-i has been found to be moderately-correlated with SDNN ($r = 0.716$, $p < 0.001$) as well as with SDANN ($r = 0.580$, $p < 0.001$), indicating that men have higher dynamic complexity of heart rate compared with women which is confirmed by the higher SDNN-i value demonstrated in men. Besides of being highly-associated with SDNN and SDANN, SDNN-i is most closely related to VLF power over a 24-hour period (Shaffer and Ginsberg, 2017). It should be noted that, even though there were some papers outlined that efferent sympathetic activity contributes to the development of VLF power as this component is predominantly subjected to the heart's intrinsic nervous system that is believed to be sympathetically-driven when there is a significant stressor (Shaffer, McCraty and Zerr, 2014; Shaffer and Ginsberg, 2017), activation of parasympathetic nervous control may promotes the generation of VLF power. This finding is suggested in the publication of Taylor *et al.* (1998) and Shaffer and Ginsberg (2017) who suggested that the VLF power could be almost completely diminished if the parasympathetic activation is obstructed. Thus, this outcome may unmask the truth that men are predominantly dependent on the parasympathetic modulation.

Parasympathetically-mediated rMSSD value which is highly correlated with the HF power frequency-domain measure, is significantly higher in men compared to women, indicating that men possess greater vagal tone which in turns, unveil the predominance of sympathetic and parasympathetic regulation in female and male subjects respectively. A significant lower value for all HRV measures found in women could have further unveiled the fact that, women possess with lower parasympathetic activity which is supported by a conspicuous higher heart rate profile and a notable lower rMSSD. This finding is upheld by previous researchers who demonstrated that higher heart rate will definitely lead to lower HRV as presented in the section 2.5.3. In short, a

strong cardiac autonomic nervous system required the interplay of sympathetic and parasympathetic modulation. In this regard, men are notably surpassed women in regulating the heart rate fluctuations that is primarily parasympathetically-controlled.

4.3.2 Effects of Aging

Table 4.6 shows the results of two-tailed Pearson's r correlation test to determine the relationship between age and time-domain HRV indexes. There was significant evidence in which, a relationship was exhibited between age and SDNN ($r = -0.214$, $p < 0.001$), SDANN ($r = -0.186$, $p < 0.001$), SDNN-i ($r = -0.336$, $p < 0.001$) and rMSSD ($r = 0.105$, $p < 0.001$). Overall HRV parameters were found to be negatively-correlated with age, with the only notable exception of rMSSD in which, a positively weak association was established with age ($r = 0.105$, $p < 0.001$), concluding that there were significant changes on HRV with increasing of age.

Table 4.6: Correlation Between Age and 24-hour HRV Parameters

Time-domain HRV index	**Correlation Coefficient (r)**
SDNN (ms)	-0.214^{**}
SDANN (ms)	-0.186^{**}
SDNN-i (ms)	-0.336^{**}
rMSSD (ms)	0.105^{**}

** $p < 0.001$; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-min duration; rMSSD = root-mean-square of the successive NN intervals difference.

Table 4.7 illustrates the influences on time-domain HRV indexes with aging, concluding that all 24-hour HRV parameters manifested an approximate linear reduction with increasing age by decade, aside from vagal-mediated rMSSD. Decade-to-decade comparisons of SDNN were comparable to that of SDANN in which, both variables exhibited no significant differences between group 1 and 2 (SDNN, $p = 0.099$; SDANN, $p = 0.220$). When steeping into

twenties, a conspicuous decrease was noted for both indexes and continued a precipitous drop until the age of thirties. A more gradual reduction was reported when coming to the age of 40 years old and above in which, no remarkable declines were noticed between group 4 and 5 (SDNN: $p = 0.06$; SDANN: $p = 0.762$), group 5 and 6 (SDNN: $p = 0.639$; SDANN: $p = 0.531$), group 7 and 8 (SDNN: $p = 0.667$; SDANN: $p = 0.636$) as well. On the other hand, SDNN-i experienced rapid decrease from the first decade and came to a halt by the seventh decade with the absence of notable differences showed in between group 6 and 7 ($p = 0.936$), group 6 and 8 ($p = 0.157$) as well as group 7 and 8 ($p = 0.748$). The steep decline was then followed by a less steep slope, beginning in the age of 60 years old which is believed to be caused by the insignificant decrease of SDNN-i, referring to the mean plots which depict the effects of aging on each HRV parameter by assessing the variations across different age groups in term of mean as illustrated in Figure 4.1.

Vagus-associated rMSSD demonstrated a U-shaped pattern unlike negatively linear relationship with age as seen for SDNN, SDANN and SDNN-i. The parasympathetic outflow, determined using rMSSD, reported no notable decrease between group 1 and 2 ($p = 0.130$) , followed by an instant drop with aging in the first instance. Global reduction of rMSSD was conspicuously observed to be initiated from the third until the sixth decade, which the rMSSD was at rock bottom. The low-water mark then ceased at the sixth decade in which, the decreasing trend appeared to be abruptly reversed itself with an accelerated increase of rMSSD was observed in the later life. This reversal was likewise indicated by the results of post hoc test which revealed there were no pronounced differences in teenagers aged below 20 and elderly aged 80 and above ($p = 1.000$), together with juniors aged 20 – 29 and seniors aged within the range of 70 – 79 years ($p = 0.973$).

Table 4.7: Effects of Aging on 24-hour Time-domain HRV Parameters

Group	Mean ± s.d.			
	SDNN (ms)	**SDANN (ms)**	**SDNN-i (ms)**	**rMSSD (ms)**
1	163.35 ± 42.55 [c,d,e,f,g,h]	143.11 ± 42.24 [c,d,e,f,g,h]	73.13 ± 19.58 [b,c,d,e,f,g,h]	50.24 ± 20.73 [c,d,e,f,g]
2	153.58 ± 41.39 [c,d,e,f,g,h]	134.86 ± 39.58 [c,d,e,f,g,h]	68.13 ± 19.33 [a,c,d,e,f,g,h]	44.27 ± 19.15 [c,d,e,f,h]
3	146.05 ± 40.55 [a,b,d,e,f,g,h]	128.50 ± 39.11 [a,b,d,e,f,g,h]	63.91 ± 18.36 [a,b,d,e,f,g,h]	39.19 ± 17.58 [a,b,d,e,f,g,h]
4	135.82 ± 38.22 [a,b,c,f,g,h]	120.23 ± 35.95 [a,b,c,f,g,h]	57.25 ± 17.57 [a,b,c,e,f,g,h]	33.86 ± 16.11 [a,b,c,e,f,g,h]
5	131.55 ± 39.17 [a,b,c,g,h]	118.04 ± 37.76 [a,b,c,g,h]	51.17 ±15.79 [a,b,c,d,f,g,h]	32.13 ± 16.66 [a,b,c,d,f,g,h]
6	129.46 ± 37.89 [a,b,c,d,g,h]	115.85 ± 36.12 [a,b,c,d,g,h]	49.46 ± 15.93 [a,b,c,d,e]	36.84 ± 19.53 [a,b,c,d,e,g,h]
7	125.95 ± 35.91 [a,b,c,d,e,f]	112.07 ± 34.79 [a,b,c,d,e,f]	48.90 ± 15.71 [a,b,c,d,e]	44.47 ± 22.02 [a,c,d,e,f,h]
8	123.63 ± 35.62 [a,b,c,d,e,f]	109.78 ± 34.77 [a,b,c,d,e,f]	47.96 ± 15.77 [a,b,c,d,e]	49.83 ± 22.78 [b,c,d,e,f,g]
Total	**132.25 ± 38.93****	**117.65 ± 37.16****	**52.86 ± 17.68****	**39.72 ± 20.45****

** $p < 0.001$ (conducted with One-Way ANOVA); s.d. = standard deviation; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-minute duration; rMSSD = root-mean-square of the successive NN intervals difference.

Tukey's Post Hoc Test Results: [a] $p < 0.05$ for other groups versus Group 1; [b] $p < 0.05$ for other groups versus Group 2; [c] $p < 0.05$ for other groups versus Group 3; [d] $p < 0.05$ for other groups versus Group 4; [e] $p < 0.05$ for other groups versus Group 5; [f] $p < 0.05$ for other groups versus Group 6; [g] $p < 0.05$ for other groups versus Group 7; [h] $p < 0.05$ for other groups versus Group 8.

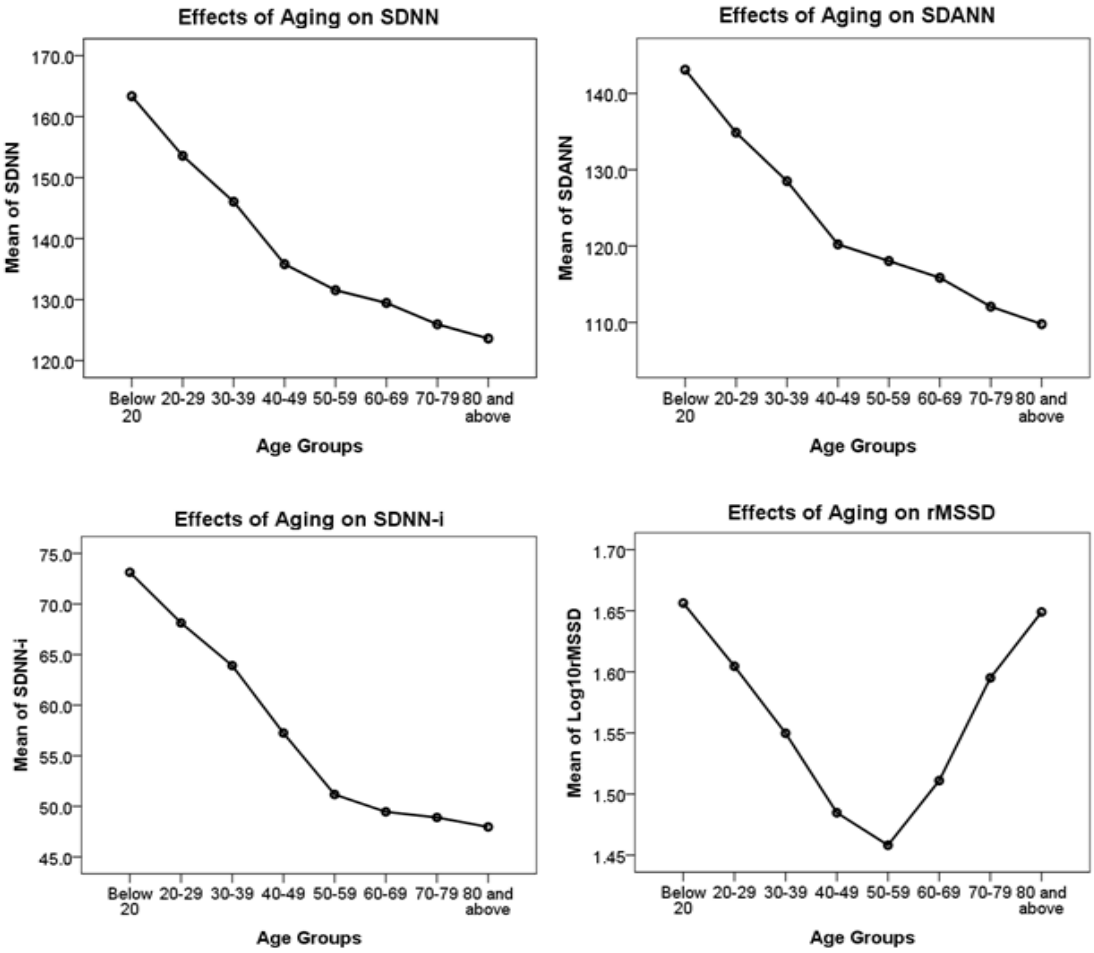


Figure 4.1: Mean Plots of Aging Effects on 24-hour Time-domain HRV Parameters

It has been well-recognised that SDNN and SDANN served as prognosticators indicating the overall complexity of heart rate dynamics in clinical settings. Thus, global reductions of these time-domain HRV indexes disclosed the fact of deterioration of overall cardiac autonomic nervous control with normal aging. The results of SDNN, SDANN and SDNN-i are consistent with earlier findings in which, negative correlations with age were reported suggesting that the degeneration of cardiac autonomic modulation is age-related (Stein, Kleiger and Rottman, 1997; Umetani *et al.*, 1998; Ramaekers *et al.*, 1998; Bonnemeier *et al.*, 2003; Antelmi *et al.*, 2004; Beckers, Verheyden and Aubert, 2006; Zulfiqar *et al.*, 2010; Abhishekh *et al*, 2013).

The data show that the HRV ruled by SDNN and SDANN exhibited weak correlations with increasing of age, indicating an age-associated decrement in a gradual pattern over time. This finding is keeping in line with the study of Bigger *et al.* (1995) and Umetani *et al.* (1998) by concluding that

aging affected SDNN and SDANN the least. The only notable exception to the progressive trend is found for subjects aged below 40 years old in which, an accelerated decline is demonstrated from third decade until the fifth decade. Perhaps not surprisingly, having different levels of activity between teenagers and elderly could have explained the situation (Umetani *et al.*, 1998; Bonnemeier *et al.*, 2003; Sandercock, Bromley and Brodie, 2005; Beckers, Verheyden and Aubert, 2006).

Using SDNN-i measure, it demonstrates the strongest correlation with increasing age among all the HRV measures ($r = -0.336$, $p < 0.001$). This result is in accordance with publications of Umetani *et al.* (1998) and Bonnemeier *et al.* (2003) in which, the former one yielded a regression power of -0.63 ($p < 0.05$) whilst the latter one returned a correlation coefficient of -0.64 ($p < 0.001$) for SDNN-i. To date, there have no definite philosophies behind the SDNN-i. Previous studies based on spectral analysis have suggested that the SDNN-i is highly-correlated with VLF and LF powers, however, the mechanisms that prompted the VLF and LF components are yet obscure. Taylor *et al.* (1998) suggested that presence of parasympathetic outflow induced a significant change on VLF power. While for LF power, Reyes del Paso *et al.* (2013) and Shaffer, McCraty and Zerr (2014) postulated that LF power is common in the clinical practice to assess the complex interplay of both sympathetic and vagal regulation but predominately by the latter one in which, a notable decrease in LF component is observed when there is a vagal blockade whereas sympathetic blockade does not yield significant effect. The unspectacular decrease in the SDNN-i from the seventh to tenth decade could have indicated that an almost imperceptible reduction of parasympathetic activity in the later life which is likewise supported by the significant improvement of parasympathetically-driven rMSSD beyond age of 60.

On the other hand, vagal-mediated rMSSD provides conflicted result which is in opposition to most of the findings outlined from the earlier studies that revealed the relationship between parasympathetic modulation and aging (Stein, Kleiger and Rottman, 1997; Umetani *et al.*, 1998; Ramaekers *et al.*, 1998; Bonnemeier *et al.*, 2003; Madden, Levy and Stratton, 2008; Berntsen *et al.*, 2011; Clancy, Deuchars and Deuchars, 2013). Unlike a linear decrease

with increasing age reported from the previous studies, rMSSD yielded a U-shaped pattern. Almeida-Santos *et al.* (2016) have supported the result by demonstrating a U-shaped plot for rMSSD. In the study of Almeida-Santos *et al* (2016), teenagers were excluded by only recruiting a total of 1743 adults and elderly aged between 40 and 100 to investigate the global autonomic regulation by means of SDANN, SDNN-i, SDNN, pNN50 and rMSSD. With all measures, HRV exhibited linear decline with aging except for rMSSD and pNN50. A notable decrease was demonstrated for both measures, beginning in the fifth decade, followed by a steady decline until the seventh decade which both rMSSD and pNN50 reached their lowest point. The rock-bottom then halted by an abrupt reversal in which, an unexpected increase was observed until the tenth decade. As suggested by Almeida-Santos *et al.* (2016), there is currently no definite explanation in terms of pathophysiological mechanism that could unmask the unanticipated reversal of parasympathetic regulation happened in the later years but perhaps, the subsequent increase of HRV-parasympathetic rMSSD measure represents a key determinant of longevity (Zulfiqar et *al.*, 2010).

4.3.3 Impacts of Gender with Aging

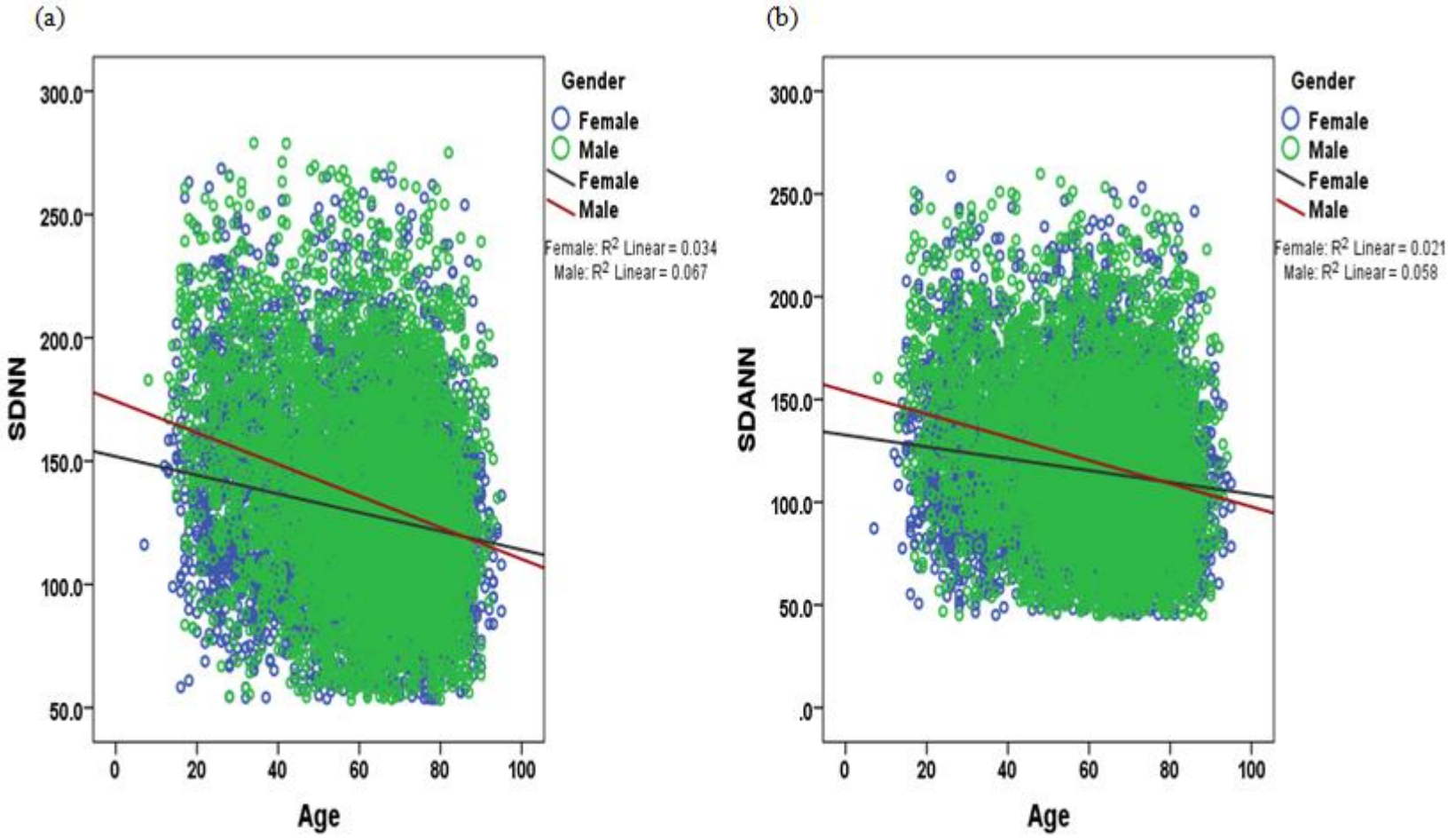
Table 4.8 demonstrates the gender distribution of study subjects by decade. There was no mean difference between males and females in all age groups with the only exception found for subjects with age of 80 years old and above, suggesting that women were significantly older than age-matched men in which, the mean age for women was about 1 year old greater than the mean age for men ($p = 0.001$).

Table 4.8: Gender Distribution of Study Subjects by Decade

Age (years)	**Total (n = 10645)**	**Gender**		**P-value**
		Male (n = 5006)	**Female (n = 5639)**	
< 20	144	71	73	0.248
20 – 29	627	211	416	0.944
30 – 39	844	346	498	0.162
40 – 49	1163	540	623	0.136
50 – 59	1734	811	923	0.688
60 – 69	2565	1285	1280	0.127
70 – 79	2360	1125	1235	0.518
≥ 80	1208	617	591	< 0.05*

* $p < 0.05$.

Figure 4.2 and 4.3 manifest the results of multiple regression analysis to provide insights into how gender affects HRV with aging. Results from multiple regression analysis unmasked the overall association between HRV and age for men and women, suggesting that only rMSSD exhibited a quadratic term which is went against the global reductions observed in SDNN, SDANN and SDNN-i measures with aging in which, the negative correlations were linearly established between HRV and age. In this regard, men demonstrated a steeper negative slope compared to the women for all HRV parameters. Furthermore, overall HRV measures were more strongly correlated with men, which were further evidenced by the higher correlation coefficients scored by male subjects than those of the female subjects with the only exception of rMSSD.



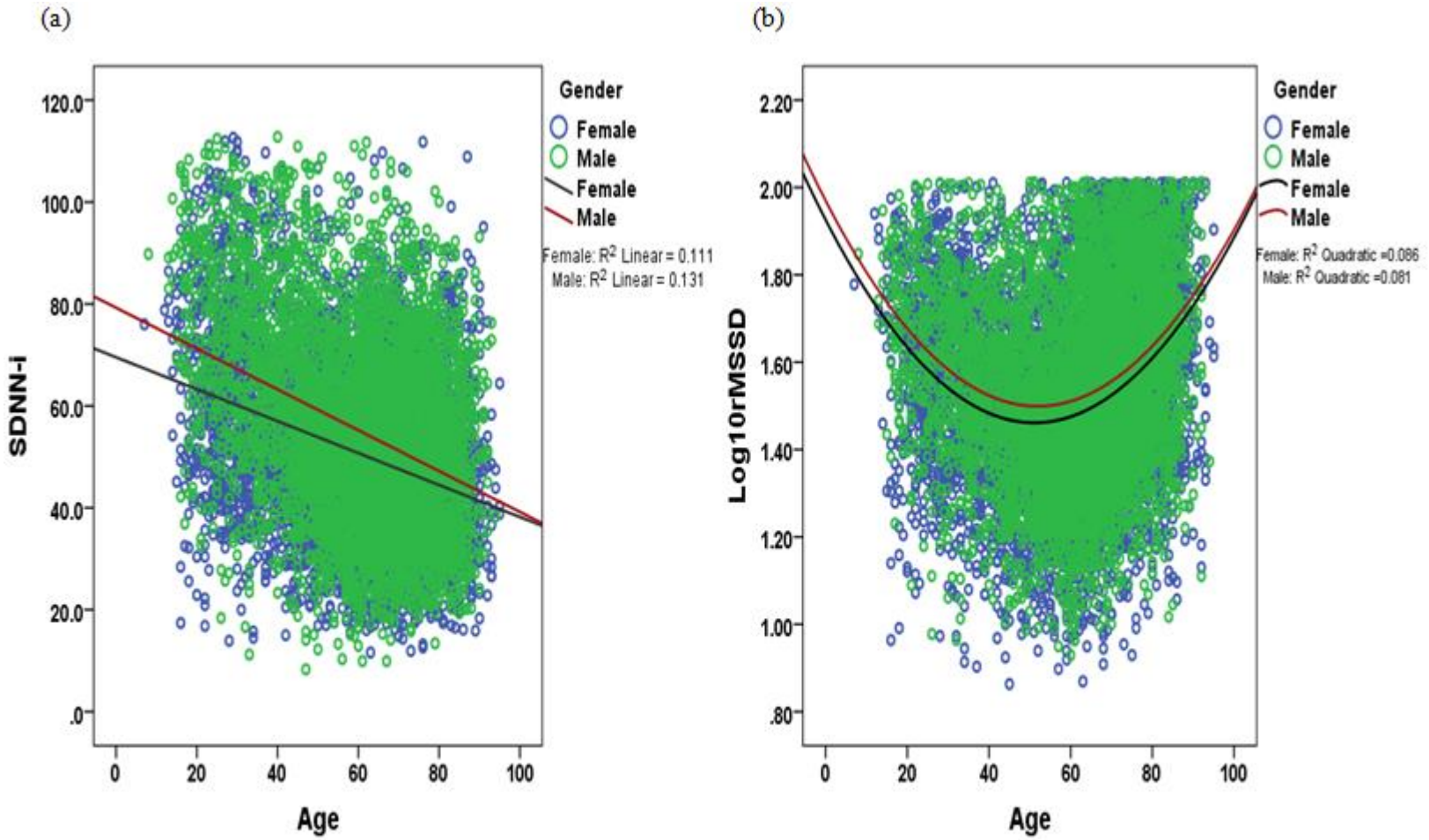
Male: y = -0.641x + 174.30 r = -0.259, p < 0.001

Female: y = -0.378x + 151.83 r = -0.184, p < 0.001

Male: y = -0.564x + 154.16 r = -0.240, p < 0.001

Female: y = -0.288x + 132.67 r = -0.146, p < 0.001

Figure 4.2: (a) Relations between Age and SDNN with respect to Gender. (b) Relations between Age and SDANN with respect to Gender.



Male: $y = -0.401x + 79.26$ $r = -0.362$, $p < 0.001$ Male: $y = 0.000175x^2 - 0.018x + 1.970$ $r = -0.285$, $p < 0.001$

Female: $y = -0.313x + 69.53$ $r = -0.333$, $p < 0.001$ Female: $y = 0.000178\ x^2 - 0.018x + 1.926$ $r = -0.293$, $p < 0.001$

Figure 4.3: (a) Relations between Age and SDNN-i with respect to Gender. (b) Relations between Age and rMSSD with respect to Gender.

Table 4.9 illustrates the main effects and interaction effects between subjects. A two-way ANOVA was performed to explore the effect of gender and aging on 24-hour time-domain HRV variables, reporting that main effects of gender and age were statistically significant for all time-domain HRV variables. Besides that, it can be observed that there was a statistical significant interaction effect for SDNN, SDANN and SDNN-i ($p < 0.001$) whereas for rMSSD, the interaction effect did not reach statistical significance ($p = 0.631$). These outcomes suggested that the effects on all HRV parameters with aging were gender-related with the only exception of rMSSD in which, the influences on rMSSD with increasing age was gender-independent and this was further confirmed by the estimated marginal means plots in Figure 4.4 which yielded two similar fluctuated trends, indicating that the effect of aging on rMSSD was consistent for both males and females.

Table 4.9: Variance Tests of Between Subjects Effects

Time-domain HRV Indexes	**P-value**		
	Gender	Age	Gender * Age
SDNN	< 0.001**	< 0.001**	< 0.001**
SDANN	< 0.001**	< 0.001**	< 0.001**
SDNN-i	< 0.001**	< 0.001**	< 0.001**
rMSSD	< 0.001**	< 0.001**	0.631

** $p < 0.001$.

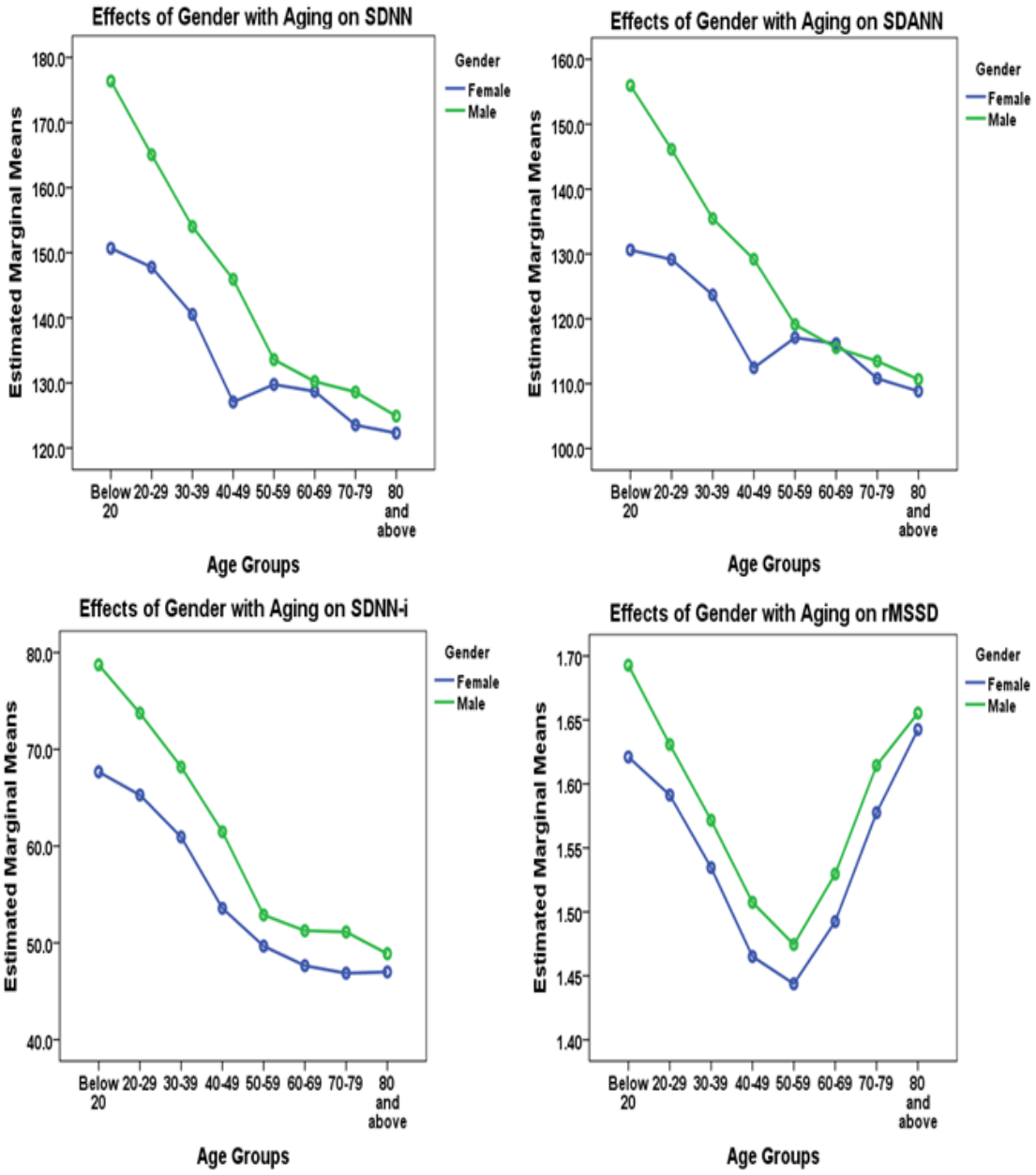


Figure 4.4: Effects of Gender with Aging on 24-hour Time-domain HRV Parameters

Table 4.10 represents the gender effects on 24-hour time-domain HRV indexes by decade. It is noted that, overall HRV measures were found to be differed vastly between men and women before age of 50. Gender differences were then eventually decreased with increasing age after the fifth decade. Among all the HRV indexes, SDANN exhibited no significant gender difference when stepping into the age of 50. On the other hand, SDNN and rMSSD demonstrated a similar trend in which, gender dependence diminished only after 80 years old. However, using SDNN-i measure, gender difference

was statistically significant for the entire life. The notable gender differences have further inferred that all HRV measures were significantly higher in male subjects compared to that of the age-matched female subjects regardless of age.

Age-specific difference in HRV was found to be more pronounced for male subjects in which, women showed a more gradual decrease for all parameters with aging. Age-dependent decrease in SDNN was comparable to SDANN for men. Comparisons between decades have revealed that the SDNN and SDANN showed significant decrease with maturity, suggesting that both variables commenced to drop conspicuously before 60 years old, followed by a more gradual decline until 70 years old for male subjects. Beyond the age of 70, SDNN and SDANN did not differed significantly, indicating that there was no discernible drop. Contrary to what have demonstrated by men, HRV determined by SDNN and SDANN did not differed significantly with respect of the comparisons between group 1 and 2, suggesting that the decrease was unobtrusive before age of 30 for female subjects. When reaching their thirties, SDNN and SDANN began to decrease significantly but this decline only persisted until the fifth decade. The decreasing trends for SDNN and SDANN in female subjects were abruptly interrupted by a significant increase at the age of 50 - 59. Linear pattern of decline was then continued, beginning from seventh decade until the tenth decade.

Similar to SDNN and SDANN, global reduction of SDNN-i for male subjects was pronounced until the seventh decade and then ceased, replaced by a more gradual decline until the tenth decade. In opposition to what have been discovered in men, women demonstrated a notable drop in SDNN-i, starting from their thirties and halted at the seventh decade. There was no significant difference beyond age of 70, implying that the female seniors with the age of 70 years old and above would have comparable SDNN-i. Using rMSSD measure, the effect of aging did not differed between men and women. Linear decrease was significant, starting in the first decade and third decade for men and female respectively, then ceased before 60 years old. Negative correlated pattern of rMSSD for both genders was then suddenly experienced reversal at their sixties, followed by an unexpected increase in the later life.

Table 4.10: Gender Effects on 24-hour Time-domain HRV Indexes by Decade

Age (years) and Gender	Mean ± s.d.			
	SDNN (ms)	SDANN (ms)	SDNN-i (ms)	rMSSD (ms)
< 20				
M	* 176.37 ± 40.60 b,c,d,e,f,g,h	* 155.95 ± 41.20 b,c,d,e,f,g,h	* 78.73 ± 18.38 b,c,d,e,f,g,h	* 52.39 ± 17.72 b,c,d,e,f,g
F	150.69 ± 40.79 c,d,e,f,g,h	130.61 ± 39.66 d,e,f,g,h	67.67 ± 19.28 c,d,e,f,g,h	48.15 ± 23.22 c,d,e,f
20 – 29				
M	* 165.05 ± 41.07 a,c,d,e,f,g,h	* 146.12 ± 40.07 a,c,d,e,f,g,h	* 73.74 ± 19.68 a,c,d,e,f,g,h	* 46.88 ± 19.61 a,c,d,e,f
F	147.77 ± 40.37 c,d,e,f,g,h	129.15 ± 38.12 c,d,e,f,g,h	65.28 ± 18.54 c,d,e,f,g,h	42.95 ± 18.80 c,d,e,f,h
30 – 39				
M	* 154.01 ± 40.40 a,b,d,e,f,g,h	* 135.45 ± 39.81 a,b,d,e,f,g,h	* 68.18 ± 18.00 a,b,d,e,f,g,h	* 40.83 ± 17.52 a,b,d,e,f,g,h
F	140.52 ± 39.77 a,b,d,e,f,g,h	123.68 ± 37.92 b,d,e,f,g,h	60.94 ± 18.05 a,b,d,e,f,g,h	38.05 ± 17.55 a,b,d,e,f,g,h
40 – 49				
M	* 145.91 ± 40.85 a,b,c,e,f,g,h	* 129.19 ± 38.06 a,b,c,e,f,g,h	* 61.48 ± 19.08 a,b,c,e,f,g,h	* 35.55 ± 16.47 a,b,c,e,f,g,h
F	127.07 ± 33.44 a,b,c,h	112.46 ± 32.08 a,b,c,e,f	53.58 ± 15.24 a,b,c,e,f,g,h	32.40 ± 15.66 a,b,c,e,f,g,h
50 – 59				
M	* 133.58 ± 41.48 a,b,c,d,f,g,h	119.11 ± 39.64 a,b,c,d,f,g,h	* 52.88 ± 16.71 a,b,c,d,f,g,h	* 33.23 ± 16.94 a,b,c,d,f,g,h
F	129.76 ± 36.96 a,b,c,g,h	117.10 ± 36.01 a,b,c,d,g,h	49.67 ± 14.78 a,b,c,d,f,g,h	31.17 ± 16.35 a,b,c,d,f,g,h
60 – 69				
M	130.21 ± 39.65 a,b,c,d,e,h	115.54 ± 37.31 a,b,c,d,e,h	* 51.26 ± 16.84 a,b,c,d,e,h	* 38.48 ± 20.17 a,b,c,d,e,g,h
F	128.70 ± 36.03 a,b,c,g,h	116.17 ± 34.88 a,b,c,d,g,h	47.65 ± 14.74 a,b,c,d,e	35.20 ± 18.72 a,b,c,d,e,g,h
70 – 79				
M	* 128.60 ± 38.19 a,b,c,d,e	113.47 ± 36.67 a,b,c,d,e	* 51.13 ± 16.33 a,b,c,d,e,h	* 46.35 ± 22.42 a,c,d,e,f,h
F	123.53 ± 33.54 a,b,c,e,f	110.79 ± 32.95 a,b,c,e,f	46.87 ± 14.85 a,b,c,d,e	42.76 ± 21.52 c,d,e,f,h
≥ 80				
M	124.91 ± 37.50 a,b,c,d,e,f	110.67 ± 36.78 a,b,c,d,e,f	* 48.88 ± 15.26 a,b,c,d,e,f,g	50.05 ± 21.87 c,d,e,f,g
F	122.29 ± 33.53 a,b,c,d,e,f	108.85 ± 35.17 a,b,c,e,f	47.00 ± 16.25 a,b,c,d,e	49.60 ± 23.72 b,c,d,e,f,g

s.d. = Standard Deviation; SDNN = standard deviation of all NN intervals over 24-hour; SDANN = standard deviation of the means of all sequential 5-minute NN intervals; SDNN-i = mean of standard deviation of total NN intervals for all 5-min duration; rMSSD = root-mean-square of the successive NN intervals difference.

Tukey's post hoc test results:

* $p < 0.05$ for male (M) versus female (F) in same age range, [a] $p < 0.05$ for other groups versus Group 1 for same gender; [b] $p < 0.05$ for other groups versus Group 2 for same gender ; [c] $p < 0.05$ for other groups versus Group 3 same gender; [d] $p < 0.05$ for other groups versus Group 4 for same gender; [e] $p < 0.05$ for other groups versus Group 5 for same gender; [f] $p < 0.05$ for other groups versus Group 6 for same gender; [g] $p < 0.05$ for other groups versus Group 7 for same gender; [h] $p < 0.05$ for other groups versus Group 8 for same gender.

The results confirmed gender effects on all HRV measures during their childhood and adolescent, demonstrating that men would have greater HRV than women. Because males score higher values for overall HRV parameters, it was suggested that men possess higher cardiac autonomic regulation that is predominantly vagal-modulated which is commonly accompanied by a further reduction of sympathetic regulation of the sinus node below age of 20. These findings are somehow keeping with those of Silvetti, Drago and Ragonese (2001), who examined whether the HRV of children and adolescence are gender-related and age-associated by recruiting 57 males and 46 females within the range of 1 – 20 years old. Unlike the results proposed in this study, Silvetti, Drago and Ragonese (2001) reported that only SDNN and SDANN differ significantly for both genders in which, a notable higher SDNN and SDANN was scored by male subjects, concluding that only SDNN and SDANN increased significantly with maturity and were gender-dependent. HRV dictated by rMSSD, pNN50 and SDNN-i rapidly increased before 10 years old, however, these differences were gender-independent.

Previous studies have postulated that gender differences in HRV are heavily dependent on age when attaining adulthood (20 to 29 years, 30 to 39 years and 40 to 49 years) (Ramaekers *et al.*, 1998; Umetani *et al.*, 1998;

Bonnemeier *et al.*, 2003; Yukishita *et al.*, 2010). Bonnemeier *et al.* (2003) describe the gender effects by means of SDNN, SDANN, SDNN-i, rMSSD and pNN50, suggesting that SDNN-i and rMSSD were significantly higher in men (20 to 29 years) whereas for subjects within age range of 40 to 49 years old, a conspicuous higher SDANN and SDNN-i were observed for men. All measures showed significant negative correlations with aging. In contrast to some of the results suggested by Bonnemeier *et al.* (2003), the data suggested that overall HRV measures linearly decrease during the transition from adolescence to adulthood and the differences between men and women are significant. These gender-related differences of cardiac autonomic nervous control with higher HRV of male subjects compared with age-matched female subjects have been reported in this study, indicating that men continue outshine women by having greater capability to regulate heart rate automatically with higher parasympathetic activity found in men.

Although there are many findings concluded that gender differences on 24-hour HRV diminished approximately at the age of 40 to 50 years in which, HRV of men were comparable to the women (Stein, Kleiger and Rottman, 1997; Ramaekers *et al.*, 1998; Umetani *et al.*, 1998; Agelink *et al.*, 2001; Bonnemeier *et al.* 2003), the results in this study exhibited contradictory behaviour by demonstrating only SDANN showed no gender difference beyond age of 50. In contrast to SDANN, men aged 50 years old and above are remarkably having higher SDNN, SDNN-i and rMSSD compared to the age-matched women. Besides that, it should be noted that, while men demonstrate a continuous decrease in all the HRV parameters, women show an unexpected increase for overall HRV measures with the only exception of SDNN-i and rMSSD at the sixth decade, but then continued with a progressive decrease began in the seventh decade. The sudden improvement on HRV in female subjects could have attributed to the episode of menopause which is generally happened after age 45. During menopause, the production of female sex hormones such as estrogen and progesterone will decrease sharply. Since estrogen is believed to be one of the contributors that result in a greater vagal tone in women (Liu, Kuo and Yang, 2003; Bonnemeier *et al.*, 2003; Beckers, Verheyden and Aubert, 2006; Yukishita *et al.*, 2010), an abrupt depression could have prompted a significant higher level of sympathetic activities and

thus, eventually indirectly lead to an improvement of global autonomic function. A lower parasympathetic activity in female subjects aged within 50 - 59 years old supports this prospect.

Studies on gender influence with respect of age have outlined that women are known to outlive men as females tend to develop cardiovascular disease at a later age than men. Perhaps not surprisingly, it is due to the long-term exposure to estrogen (Stein, Kleiger and Rottman, 1997; Ramaekers *et al.*, 1998; Umetani *et al.*, 1998; Yukishita *et al.*, 2010). The results agree with the findings in most studies, suggesting that women would have experienced more gradual decline in global autonomic function in respect of age in which, a less steep slope is observed for all HRV variables. Besides that, women showed more instant improvement in vagal-mediated rMSSD at the later age, further confirming the truth that women are generally outshined men in term of longevity as higher parasympathetic activity has been found to be associated with the reducing vulnerability to lethal arrhythmias and to the development of coronary heart disease (Ramaekers *et al.*, 1998; Umetani *et al.*, 1998; Yukishita *et al.*, 2010).

4.4 Summary

In this section of the study, the influences of age and gender on 24-hour time-domain HRV measures were determined by comparing the results based on previous investigations. In general, none of the HRV parameters were higher in women, indicating that men are excelled at cardiac autonomic regulation with higher level of parasympathetic activity compared with women. This result is keeping with some of the publications as gender-HRV relations are yet ambiguous which required further studies.

All HRV measures showed negative correlated pattern with age similar to what have been published in recent studies except rMSSD in which, a U-shaped plot was yielded. The continuous decline in HRV-parasympathetic function was halted at the sixth decade which then replaced by a significant increase to higher levels, suggesting that a lower risk to the arrhythmic death, against the other death modes at the later age and perhaps the decisive factor in achieving higher life expectancy, however, the philosophy behind the reversal is unknown and thus, future works are necessary.

With increasing of age, males and females affected overall HRV variables in their particular way with the only notable exception of rMSSD, demonstrating that the influence on rMSSD of men is comparable to that of the women throughout the lifetime. As suggested by existing researches, the gender differences on HRV were significant and these differences were slowly decreased with increasing age after fifth decade, suggesting that men possess higher HRV than age-matched women. It has been observed that SDANN is the only HRV parameter which supports the view of previous researches, demonstrating that the gender difference is diminished at 50 years old. The reversal happened in female subjects at age 50 - 59 for SDNN and SDANN in which, a negative correlated pattern reversed itself, possibly due to the non-facilitation of estrogen during menopause. A more gradual decline in total autonomic and parasympathetic functions in women with more rapid increase in parasympathetically-mediated rMSSD in later life may account for the cardioprotection in women which granted them a greater longevity than men.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Throughout the statistical analysis conducted on the data collected, the effects of age on 24-hour time-domain HRV measured have been determined, concluding that there was a significant linear pattern of decrease in all HRV measures with increasing of age except for parasympathetically-mediated rMSSD which yielded a U-shaped pattern with aging. Healthy aging is generally associated with significant impairment of autonomic nervous system, similar to what have been outlined by previous researches on age-related changes concerning matters of cardiac autonomic regulation throughout the life span. The subsequent increase of rMSSD at the later age might have contributed to the longevity.

Besides that, the study came to the conclusion that gender influences on HRV were significant, demonstrating that none of the parameters were higher in women. This is further unmasked the fact that men are having higher cardiac autonomic regulation in which, the parasympathetic activity is more pronounced while women are generally more sympathetically-regulated. Furthermore, the gender effects with maturity are likewise described in this research, unveiling that the gender differences were age-dependent for SDNN, SDANN and SDNN-i only. The gender differences did not diminished at the age of 40 or 50 with the only exception of SDANN, in contrast to what have been suggested in the existing publications. Using SDNN and SDANN measures, women exhibited an unexpected increase at the sixth decade further highlighting that the importance of estrogen. Women might be more cardioproctective than men which awarded them a greater longevity that are reflected by a more gradual depression in all HRV measures and a more instant improvement in rMSSD in their later life.

5.2 Recommendations for Future Work

In order to improve the prognostic value of HRV in clinical settings, it is strongly suggested that the researchers should collect more details from subjects. Information about sleep are one of the noteworthy particulars that should be acquired. This can be done by performing polysomnography on subjects to allow the interpretation of circadian patterns. Besides that, future studies may have work on the data acquisition in terms of the subjects' activity level and medical background. This is due to the fact that both the physical fitness and health condition could have affected HRV in their particular ways which in turn, made them the factors that cannot be neglected.

It should be noted that, although aging effects on HRV did not much contrasted with existing publications, the underlying causes of abrupt increase in vagal-mediated rMSSD beyond the age of 60 are yet to be attained. Besides that, the gender effects on HRV are still remained indefinite. This is partly because of the controversy surround the performance of autonomic regulation in both genders in which, some of the findings outlined that men possess higher HRV than women whereas some of the researchers opposed the view by stating that women demonstrated a higher HRV compared to that of the men. The persistence of gender difference in HRV affected by SDNN, SDNN-i and rMSSD conflicted with the findings of earlier studies which demonstrated a nearly disappearance of gender dependence at the age of 40 or 50 for all HRV measures. Thus, it is recommended that findings on HRV concerning about the age and gender impacts should be focused on the clarification of age-rMSSD associations and gender-HRV relations.

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APPENDICES

APPENDIX A: Gantt Charts

Table A-1: Gantt Chart for Phase 1 Research

No.	Project Activities	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14
M1	Problem Formulation & Project Planning														
M2	In-Depth Research														
M3	Input Data Acquaintance														
M4	Preliminary Analysis / Evaluation														
M5	Report Writing & Submission														

Table A-2: Gantt Chart for Phase 2 Research

No.	Project Activities	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14
M1	Problems Troubleshooting														
M2	Data Analysis and Interpretation														
M3	Poster Design and Submission														
M4	Final Report Writing and Submission														