DEVELOPMENT OF STRESS BIOMARKERS THROUGH PHYSIOLOGICAL ANALYSIS USING MATLAB SOFTWARE

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UNIVERSITI TEKNIKAL MALAYSIA MELAKA

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This report is submitted in partial fulfilment of the requirements for the degree of Bachelor of Electronics Engineering Technology with Honours

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DEDICATION

To my beloved mother, Puan Sarinah, and father, Encik Abdul Razak,

and

To dearest friend, Yaya, I owe so much to you and I am truly grateful for everything.

ABSTRACT

Stress is one of the most common contributors to mental illnesses, affecting people worldwide. Recent studies have highlighted the potential of electrocardiogram (ECG) signals as biomarkers for stress detection, offering a non-invasive approach to identifying and managing these conditions. While ECG signaling characteristics have demonstrated high accuracy in identifying biomarkers, existing methods for biomarker identification remain limited. This project aims to study ECG signal biomarkers most likely to be influenced by stress, develop stress detection through ECG biomarkers using baseline methods and Bazett's formula in MATLAB, and analyze stress episodes in QT intervals of ECG signals using statistical approaches. The project employs the KL75001 ECG module and K&H software for ECG data acquisition. Mental arithmetic and the Stroop Color Word Test (SCWT) were used as stimuli to induce changes in ECG waveforms under stress conditions. QT intervals were calculated using Bazett's formula and baseline methods, while statistical analysis involving mean, variance, and standard deviation was conducted to quantify signal characteristics during stress episodes. The findings show that a QRT peak detection method was successfully implemented, enabling stress detection through ECG signals. Using Bazett's formula, it was determined that QTc intervals exceeding the baseline threshold of 0.45 indicate stress with accuracy of 70%. Positive skewness in QT intervals suggests a longer tail on the right side of the distribution, indicating more variability and a tendency toward longer QT intervals which means the data are under stress conditions. Additionally, high kurtosis reflects a distribution with heavy tails and a sharp peak, implying that extreme QT interval values, both long and short, occur more frequently under stress condition. The integration of controlled stimuli, baseline thresholding methods, and computational tools in MATLAB demonstrates the potential of this approach for applications in health monitoring, stress management, and personalized interventions aimed at mitigating stress-related health risks.

ABSTRAK

Tekanan adalah salah satu penyumbang paling biasa kepada penyakit mental, yang menjejaskan orang di seluruh dunia. Kajian terbaru telah menyerlahkan potensi isyarat elektrokardiogram (ECG) sebagai biomarker untuk pengesanan tekanan, menawarkan pendekatan bukan invasif untuk mengenal pasti dan mengurus keadaan ini. Walaupun ciri isyarat ECG telah menunjukkan ketepatan yang tinggi dalam mengenal pasti biomarker, kaedah sedia ada untuk pengenalan biomarker kekal terhad. Projek ini bertujuan untuk mengkaji biomarker isyarat ECG yang berkemungkinan besar dipengaruhi oleh tekanan, membangunkan pengesanan tekanan melalui biomarker ECG menggunakan kaedah garis dasar dan formula Bazett dalam MATLAB, dan menganalisis episod tekanan dalam selang QT isyarat ECG menggunakan pendekatan statistik. Projek ini menggunakan modul KL75001 ECG dan perisian K&H untuk pemerolehan data ECG. Aritmetik mental dan Ujian Kata Warna Stroop (SCWT) digunakan sebagai rangsangan untuk mendorong perubahan dalam bentuk gelombang ECG di bawah keadaan tekanan. Selang QT dikira menggunakan formula Bazett dan kaedah asas, manakala analisis statistik yang melibatkan min, varians, dan sisihan piawai telah dijalankan untuk mengukur ciri isyarat semasa episod tekanan. Penemuan menunjukkan bahawa kaedah pengesanan puncak QRT telah berjaya dilaksanakan, membolehkan pengesanan tekanan melalui isyarat ECG. Menggunakan formula Bazett, telah ditentukan bahawa selang QTc melebihi ambang garis dasar 0.45 menunjukkan tekanan dengan ketepatan 70%. Kecondongan positif dalam selang QT menunjukkan ekor yang lebih panjang di sebelah kanan taburan, menunjukkan lebih banyak kebolehubahan dan kecenderungan ke arah selang QT yang lebih panjang yang bermaksud data berada di bawah keadaan tekanan. Selain itu, kurtosis tinggi mencerminkan taburan dengan ekor berat dan puncak yang tajam, membayangkan bahawa nilai selang QT yang melampau, panjang dan pendek, berlaku lebih kerap dalam keadaan tekanan. Penyepaduan rangsangan terkawal, kaedah ambang asas dan alat pengiraan dalam MATLAB menunjukkan potensi pendekatan ini untuk aplikasi dalam pemantauan kesihatan, pengurusan tekanan dan campur tangan yang diperibadikan yang bertujuan untuk mengurangkan risiko kesihatan berkaitan tekanan.

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LIST OF SYMBOLS

μ	-	Mean
σ	-	Standard Deviation
σ^2	-	Variance

LIST OF ABBREVIATIONS

HR	-	Heart Rate
ECG	-	Electrocardiogram
HRV	-	Heart Rate Variability
SCWT	-	Stroop Color Word test
AI	-	Artificial Intelligence
SDG	-	Sustainable Development Goals

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Electrocardiogram (ECG) is a valuable tool for measuring the electrical activity of the heart. Studies have shown that ECG signals, along with respiration (RSP) signals, can be utilized as physiological measures for detecting stress [1]. Heart-rate variability (HRV) and breathing-rate variability (BRV) have emerged as prominent biomarkers for anxiety detection through ECG analysis. These biomarkers, such as mean heart rate (MHR), standard deviation of heart rate (SD), and standard deviation of NN intervals (SDNN), offer insights into physiological changes associated with stress states. The use of HRV and BRV as biomarkers provides a foundation for real-time data analysis and interpretation of ECG and RSP signals collected through portable and wearable devices [2]. These biomarkers enable the objective assessment of mental-emotional states, paving the way for personalized interventions and treatment strategies tailored to individual stress profiles. Prolonged or unattended stress states in an individual can lead to a new problem which is the development of anxiety. According to the World Health Organization (WHO), An estimated 4% of the global population currently experience an anxiety disorder. In 2019, 301 million people in the world had an anxiety disorder, making anxiety disorders the most common of all mental disorders. Through ECG activity analysis, physiological biomarkers are established in the management of stress and anxiety that aid the assessment of physiological changes due to emotional changes. It can also help improve the formation of correct and effective methods for identifying stress, which can in turn improve mental health.

1.2 Addressing Societal Issue From Anxiety And Stress

When stress persists or remains unattended, it can evolve into anxiety disorders or lead to depression. It is estimated that 4% of the global population currently experience an anxiety disorder. In 2019 alone, 301 million people in the world had an anxiety disorder, making anxiety disorders the most common of all mental disorders. Women are reported to be more affected by anxiety disorders than men. Individuals with anxiety and stress can have significant effects on society. Chronic stress can lead to decreased productivity, increased absenteeism, and lower job performance, ultimately affecting the overall efficiency of organizations and the economy. Furthermore, stress can contribute to the development of various chronic diseases, such as heart disease, vascular disease, and cancer, which increase healthcare costs and burden the healthcare system. Anxiety can also lead to avoidance of social interactions, which can negatively impact personal and professional relationships, ultimately affecting social cohesion and community dynamics. Biomarkers such as ECG activities can facilitate the detection of anxiety and stress disorders in their early stages, allowing for timely treatment and prevention of these conditions getting exacerbated by becoming more severe usually leading to mental health disorders. By knowing the idiosyncratic physiological responses through the process of ECG, healthcare providers are able to develop personalized treatment plans which directly target each individual's unique needs. As the technology progresses and the development of wearable and portable devices align, it also goes with the biomarkers of anxiety and stress, thanks to the continuous monitoring of individuals' physiological responses in real-time and remote monitoring and personalized interventions. Therefore, the application of the biomarkers for anxiety and stress attainable through the ECG activity would play a very important role in the early detection, individualized treatment, and overall handling of the widespread mental health afflictions within the society.

1.3 Problem Statement

Stress are some of the most common contributor that leads to mental illnesses which affect people around the globe. Since it is critical to note that these conditions need to be accurately diagnosed in order to properly intervene and effectively manage them, self-report questionnaires are certainly useful in diagnosing stress, but are not fully reliable or accurate as other types of testing. Therefore, there is a growing interest in utilizing physiological biomarkers, particularly those derived from ECG activity analysis, to provide objective and quantitative data for stress assessment [3]. Building physiological biomarkers based on the ECG activity analysis is very important as it can become a basis for creating an effective way to transform the diagnosis and treatment of stress which is a leading medical problem. Despite ECG signaling characteristics have demonstrated high accuracy for identifying biomarkers, manual analysis, lack of standardization, and individual differences in autonomic nervous system (ANS) responses create existing biomarkers identification methods limited [4]. The theme of the work is the efficiency of the ECG activity analysis in finding physiological biomarkers of stress and affection states, helped by the accuracy and objectivity of the method, eventually, used for providing personalized stress management strategies.

1.4 Project Objective

The main aim of this project is to analyse how ECG activities are affected during periods of stress and anxiety through the analysis of ECG activities, utilizing non-invasive and convenient ECG sensors placed on the wrist and ankle. The project objectives are as below:

a) To study the ECG signals biomarkers that are most likely to be influenced by stress.

b) To develop stress detection through ECG biomarkers using baseline method and Bazzett's formula in MATLAB

c) To analyse the stress episodes between QT interval for ECG signal through statistical approach.

1.5 Scope of Project

The scope of this project is as follows:

a) Carrying out controlled experiments where subjects are subjected to stimuli that cause stress while their ECG activity is recorded using non-invasive and easy-to-use ECG sensors on the wrist and ankle.

b) Determining the ECG signals which are most likely to be affected by stress then process the collected ECG data using MATLAB to extract relevant features that can classify stress states based on ECG patterns using baseline method and Bazzzett's formula.

c) Analysing the stress episodes using statistical analysis like measuring the mean, variance, standard deviation, skewness and also kurtosis of the QT interval.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter discuss about the correlation of stress and anxiety with ECG signal activities. Stress and anxiety are two related mental health conditions that can have significant impacts on an individual's well-being. Anxiety is a constant sensation of concern or dread that can interfere with day-to-day living, whereas stress is a reaction to demands or pressures from the outside world. These psychological responses can effect ones body mentally and physically. It will also affect the individual's HRV which is relatively new method for evaluating stress. HRV readings can be measured using ECG. An ECG is a non-invasive test that captures the electrical activity of the heart and can detect abnormalities in its rhythm. ECG equipment may identify physical indicators of stress and anxiety, such as variations in heart rate and rhythm. This chapter also explores about the development of physiological biomarkers through ECG activities analysis for stress and anxiety. By synthesizing current research findings, theoretical frameworks, and methodological approaches, this chapter aims to shed light light on advancement, challenges, and implications of utilizing ECG in stress and anxiety assessment.

2.2 Stress and Anxiety

In today's fast-paced world, stress and anxiety have become prevalent concerns affecting individuals across various demographics. From students navigating academic pressures to professionals juggling work demands, the impacts of stress and anxiety on mental and physical health are profound and far-reaching. Anxiety and stress are complex psychological states that can manifest in various forms and have different classifications depending on their duration, intensity, and underlying causes.

Stress refers to the body's response to demands or pressures, whether real or perceived, that require adaptation or coping [5]. While some level of stress is necessary for motivation and survival, chronic or overwhelming stress can have detrimental effects on physical and mental health. Stress is classified into two main types: acute stress and chronic stress. Acute stress is a short-term response to a stressor, while chronic stress is a long-term response to ongoing stressors. The psycho-neuroendocrine-immune (PNEI) system plays a crucial role in the body's response to stress, relying on stability of the normal internal environment of an organism based on homeostasis. Stress affects the neuroendocrine system, and in turn, stressors affect the neural and immune systems. The PNEI system is a complex system that involves the interaction between the central nervous system, endocrine system, and immune system [6]. Prolonged stress increases the chance of developing major health problems and incidents, including high blood pressure, heart attacks, and strokes. In addition to the serious long-term effects, short-term stress can have an impact on behavior and mental processes including decision-making, which is critical in a variety of application fields [7] .

Other than that, stress and anxiety could also significantly affect the ANS of an indidual. Numerous studies indicate a link between ANS dysfunction and anxiety disorders [8]. ANS is responsible for regulating involuntary bodily functions such as heart rate, respiratory rate, and digestion. The ANS has two divisions: the sympathetic nervous system

(SNS) and the parasympathetic nervous system (PNS). The SNS is responsible for the "fight or flight" response, which prepares the body for stressful events or emergencies[9]. In contrast, the PNS helps the body return to a calm state after a stressful event[10].Chronic stress and anxiety can lead to an over-activation of the SNS and under-activation of the PNS, resulting in an imbalance between these two systems. This imbalance, known as ANS dysregulation, can have serious implications for the body. It can cause a range of symptoms, including chronic muscle tension and pain, low mood, disinterest in activities, insomnia, digestive disturbance, and more.

Research has shown that anxiety activates the ANS, triggering the "flight or fight" response, which can express itself through various physiological symptoms such as a fast pulse, palpitations, shallow breathing, shortness of breath, chest pain/tightness, sweating, choking, headaches, insomnia, irritability, uncontrollable muscle tension/twitches, trembling, feeling faint/unreal, tingling in hands/arms/legs, tightness in throat, dry mouth, problems with speech, fear of dying, going mad, and losing control. Chronic stress and anxiety can also lead to changes in the body's hormonal responses, including the release of adrenaline, cortisol, and other stress hormones. These hormonal changes can have significant effects on various bodily systems, including the cardiovascular, respiratory, digestive, and immune systems.For example, chronic stress and anxiety can lead to an increase in heart rate and blood pressure, as well as changes in blood flow to various parts of the body. This can increase the risk of heart disease, hypertension, and other cardiovascular problems[11].

Chronic stress and anxiety can also lead to changes in respiratory rate and depth, which can exacerbate breathing problems for people with pre-existing respiratory diseases such as emphysema and chronic bronchitis[12]. In addition, chronic stress and anxiety can lead to changes in the digestive system, including changes in gut motility, secretion, and immune responses. These changes can result in various digestive symptoms, including abdominal pain, bloating, diarrhea, and constipation.

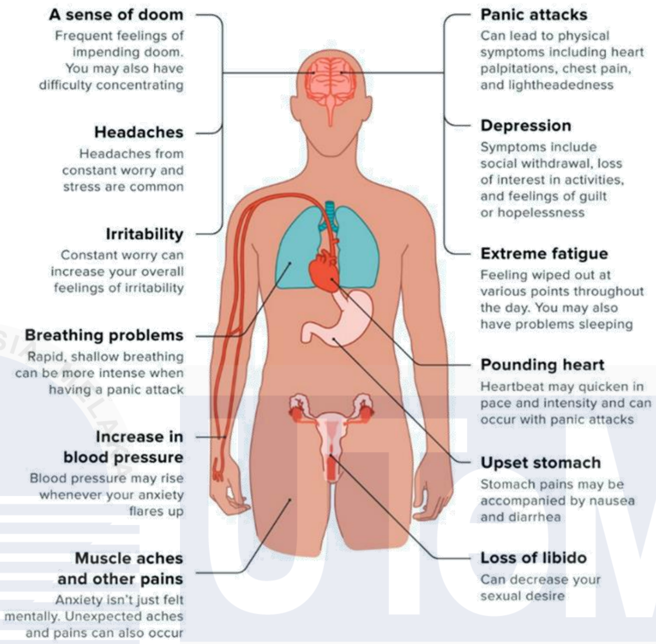

Figure 2.1 Effects of anxiety on individual

Figure 2.1 shows the effects of anxiety on individual. Anxiety and stress have a profound impact on a person's physical and mental well-being. Both conditions can produce a range of symptoms, including trouble sleeping, digestive issues, difficulty concentrating, muscle tension, irritability, and increased heart rate. Chronic anxiety can lead to long-term physical effects such as respiratory problems, gastrointestinal disorders, weakened immune systems, and increased risk of heart disease. Stress can also cause muscle tension, headaches, and changes in appetite, while anxiety can trigger panic attacks, rapid breathing, and cold hands and feet. Both conditions can disrupt daily life and negatively impact mood and functioning. If left unmanaged, they can have severe consequences on overall health and quality of life

2.2.1 Triggers and Stimulation

Triggers for anxiety and stress can vary widely and may include environmental factors, psychological stressors, and internal stimuli. Environmental triggers can encompass situations like looming work deadlines, traffic jams, or crowded places, while psychological stressors may involve persistent worries about job security, family difficulties, or financial concerns. These triggers can stimulate the body's stress response system, leading to the release of stress hormones like cortisol and adrenaline, which prepare the body for the fight or flight response.

Moreover, internal stimuli such as sensory overload can also contribute to anxiety and stress. Sensory overload, characterized by an overwhelming amount of sensory input, can be a trigger for anxiety, especially in individuals with conditions like Post-Traumatic Stress Disorder (PTSD), Generalized Anxiety Disorder, Autism, or Attention Deficit and Hyperactivity Disorder (ADHD). Understanding and managing these triggers are crucial in mitigating the impact of stress and anxiety on physical and psychological health.

The sources of stress and anxiety can be both external and internal, ranging from work-related pressures, relationship issues, financial worries, to personal insecurities and past traumas. These sources can activate the body's stress response system, leading to a cascade of physiological and hormonal changes that impact overall well-being. By recognizing and addressing these triggers and sources, individuals can take proactive steps to manage stress and anxiety effectively.

2.2.2 Physiological signal

Several medical measurement can be used to different types of physiological measurements used to monitor various aspects of bodily function. Each of these measurements can provide insights into the physiological responses associated with anxiety and stress. Physiological measurements used to monitor anxiety and stress HRV, brainwave analysis, and hormonal testing. HRV involves recording the variation in time between consecutive heartbeats, reflecting the balance of the ANS. Brainwave analysis, measured through electroencephalography (EEG), can provide insights into stress responses. Hormonal testing assesses levels of stress-related hormones like cortisol and adrenaline, which play key roles in the body's response to stress. These measurements offer valuable insights into the physiological aspects of anxiety and stress [13].

2.2.2.1 Electrocardiogram (ECG)

ECG measures stress and anxiety by analyzing HRV and other parameters derived from the ECG signal. HRV, which refers to the variation in time between consecutive heartbeats, is a key indicator of the body's response to stress and anxiety. During stressful situations, the ANS is activated, leading to changes in heart rate and HRV patterns. ECG can detect these variations in heart rate and HRV, providing insights into the individual's stress levels. Additionally, ECG can assess the sympathetic and parasympathetic nervous system activity, which are involved in the body's stress response. An increase in sympathetic activity, associated with the "fight or flight" response, and a decrease in parasympathetic activity can indicate heightened stress and anxiety levels. By monitoring these changes in the ECG signal, healthcare professionals can evaluate the impact of stress and anxiety on the cardiovascular system and overall well-being.

2.2.2.2 Electroencephalography (EEG)

EEG assesses the level of stress and anxiety by measuring brainwave activity, specifically focusing on patterns associated with stress responses. EEG analysis involves monitoring electrical activity in the brain through electrodes placed on the scalp. Different brainwave frequencies, such as beta, alpha, theta, and delta waves, are associated with various mental states, including stress and anxiety. For instance, increased beta wave activity can indicate heightened alertness or stress, while alpha waves are linked to relaxation and calmness.

By analyzing EEG signals during stress-inducing tasks or situations, researchers can identify specific patterns or changes in brainwave activity that correlate with stress levels. This information helps in understanding how the brain responds to stress and anxiety, providing insights into the physiological aspects of these conditions. Additionally, combining EEG with other physiological measurements like heart rate, blood pressure, and respiration rate can offer a comprehensive assessment of an individual's stress and anxiety levels, enhancing the accuracy of stress evaluation [14].

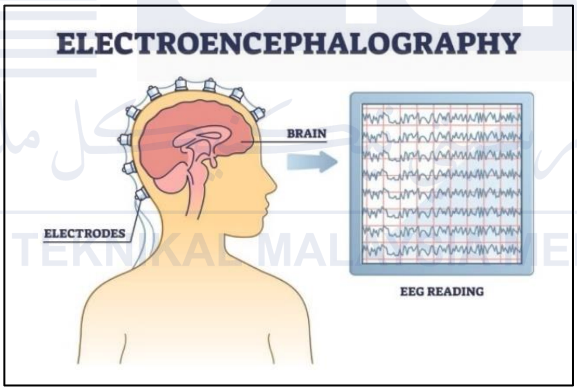

Figure 2.2 EEG placement and waveform

Figure 2.2 shows the EEG placement and waveform. EEG measures stress and anxiety by monitoring brainwave activity via scalp electrodes, focusing on patterns linked to stress. Increased beta waves indicate stress, while alpha waves suggest relaxation. Analyzing EEG during stress-inducing tasks reveals brainwave changes correlating with stress levels. Combining EEG with heart rate, blood pressure, and respiration rate provides a comprehensive and accurate assessment of an individual's stress and anxiety levels.

2.2.2.3 Photoplethysmography (PPG)

PPG assesses the level of stress and anxiety by extracting stress-related indices from the PPG signal. PPG measures changes in blood volume in the microvascular bed of tissue, typically using a light source and a photodetector. By analyzing features of the PPG signal, such as fiducial point timings, amplitudes, areas under the signals, and slopes of lines joining fiducial points, researchers can identify patterns indicative of stress and anxiety. For example, decreased timings of fiducial points on the PPG signal have been associated with stress, and certain features like CT, t, and dia IPR have shown consistent changes with stress levels across different measurement sites.

Moreover, combining multiple features extracted from the PPG signal through machine learning techniques can provide a comprehensive assessment of stress and anxiety levels. While PPG technology shows promise in assessing mental health conditions, it is essential to consider its limitations, such as the need for skilled mental health practitioners to interpret results and the importance of using it as an adjunct to clinical assessment. Understanding the physiological determinants of PPG features and their association with stress responses is crucial in utilizing PPG effectively for evaluating stress and anxiety levels [15], [16], [17].

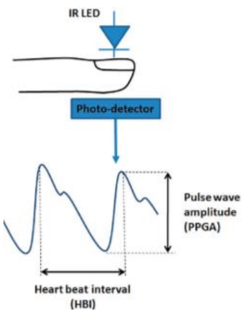

Figure 2.3 PPG Placement and waveform

While photoplethysmography (PPG) has been explored as a potential tool for measuring anxiety and stress, there are several notable limitations to its use in this context.

One of the primary challenges is the sensitivity of PPG technology to motion artifacts, which can introduce significant variability and inaccuracies in the measurements. This is particularly problematic, as anxiety and stress often manifest through physical movements and fidgeting, making it difficult to obtain reliable data.

Another limitation is the inability of PPG to reliably differentiate between different types of anxiety and stress, such as clinical anxiety, general personal stress, and common feelings of anxiety experienced by everyone. This lack of specificity makes it challenging to accurately assess the underlying causes and nature of the individual's emotional state. Furthermore, many of the studies examining the use of PPG for anxiety and stress assessment have not employed standard diagnostic methods or comparison groups, which limits the ability to evaluate the validity and reliability of this approach. This lack of comparative data makes it difficult to determine the true efficacy of PPG in this application.

Additionally, the search results indicate that it may be challenging to discern whether an individual's anxiety and stress are directly related to their mental health condition or are simply a result of general life stressors. This limitation hinders the ability to accurately attribute the measured physiological responses to specific sources of anxiety and stress. Finally, the current state of PPG technology does not allow for the assessment of the severity of symptoms or the level of functional impairment associated with anxiety and stress, which are crucial factors in clinical diagnosis and treatment planning. This limitation restricts the usefulness of PPG as a stand-alone method for comprehensive anxiety and stress evaluation.

In summary, while PPG holds promise as a non-invasive tool for monitoring physiological responses, its application in the assessment of anxiety and stress is currently limited by factors such as motion sensitivity, lack of specificity, insufficient comparative data, and the inability to determine the source and severity of the emotional states.

2.2.3 Stimulus

Several tests have been developed to trigger stress and anxiety in a controlled environment for research purposes. These tests help researchers systematically study stress and anxiety responses in a controlled setting.

2.2.3.1 Stroop Color Word Test

The Stroop Color Word Test is a cognitive task that measures an individual's ability to inhibit an automatic response. In this test, subjects are shown words representing colors (e.g., "red", "blue") printed in a different color ink (e.g., the word "red" printed in blue ink). Subjects are asked to name the color of the ink, while ignoring the word itself. The incongruence between the word and the ink color creates cognitive interference, which can be stressful and anxiety-provoking, especially when the task is timed [18].

2.2.3.2 Mental Arithmetic

Mental arithmetic tasks, such as serial subtraction or division, are commonly used to induce psychological stress in a laboratory setting. These tasks require subjects to perform complex calculations quickly and accurately, often under time pressure or social evaluation. The cognitive demand and pressure associated with these tasks can trigger stress and anxiety responses [19].

2.2.3.3 Cold Pressor Test

The Cold Pressor Test involves immersing a subject's hand or foot in ice water (usually 0-4°C) for a specified duration, typically up to 3 minutes. This physical stressor can induce pain and discomfort, leading to an acute stress response. The anticipation of pain and the inability to control the situation can also contribute to feelings of stress and anxiety [20].

2.3 ECG in anxiety and stress

The ECG is a non-invasive diagnostic tool that records the electrical activity generated by the heart. It is a graphical representation of the electrical activity of the heart, which is used to identify various heart diseases and abnormalities [21]. The ECG signal is recorded from the body surface and registers the differences in electrical potential generated by the heart. The electrical forces generated by the heart are altered by the position of the heart within the body, the nature of the intervening tissue, and the distance to the recording electrode. Therefore, the final recorded electrical signal may not faithfully reflect the electrical activity of individual cells, but it can provide an important indication of a cardiac abnormality and even allow a fairly accurate appraisal of the anatomic and physiologic significance of that abnormality.

The relationship between ECG and stress/anxiety lies in the ability of ECG to detect physiological changes in the heart that are influenced by stress and anxiety. Stress and anxiety can impact the ANS, leading to changes in heart rate, HRV, and other ECG parameters [22]. These changes can manifest as abnormalities in the ECG signal, providing insights into the individual's emotional state and its effects on the cardiovascular system.

HRV refers to the variation in the time intervals between consecutive heartbeats, as measured by an ECG. HRV is a reflection of the ANS’s influence on the heart. The ECG signal provides the necessary data to calculate various HRV parameters, both in the time domain (e.g. SDNN, RMSSD, pNN50) and frequency domain (e.g. LF, HF, LF/HF ratio). These HRV parameters provide insights into the balance between the sympathetic and parasympathetic branches of the ANS.Specifically, ECG allows for the precise measurement of the time intervals between consecutive heartbeats (R-R intervals), which is the basis for calculating HRV. Changes in HRV, as detected through ECG analysis, reflect alterations in the ANS, which can be influenced by factors like stress, anxiety, physical activity, and disease states. HRV parameters derived from the ECG signal can be used to assess cardiac autonomic function and provide insights into an individual's physiological and psychological state. Reduced HRV, as measured by ECG, has been associated with increased risk of cardiac mortality and morbidity in various patient populations.

Other than that, stress and anxiety can also cause certain ECG abnormalities, such as T-wave inversion, QT prolongation, and rhythm irregularities. These abnormalities may be associated with short-term test nervousness or chronic anxiety, affecting the interpretation of ECG results [23]. Understanding the relationship between stress/anxiety and ECG findings is crucial for healthcare providers to differentiate between cardiac issues and changes induced by emotional factors.

2.3.1 Rhythmic signal

ECG rhythmic signals are recordings of the electrical activity of the heart, which can be used to assess stress and anxiety levels [7]. The ECG signal reflects the electrical heart activity by detecting changes in the voltage on the surface of the skin due to the heart's electrical activity. The most dominant waveform in the ECG signal is the QRS waveform, also known as the QRS complex. The QRS complex is formed by the depolarization of the ventricles and is the most prominent feature in the ECG signal. During periods of stress and anxiety, the ANS is activated, leading to changes in the ECG signal. Specifically, HRV, which is a feature extracted from the ECG signal, is known to be relevant to stress and anxiety assessment. HRV indexes the cardiac vagal tone, which represents the contribution of the parasympathetic nervous system to cardiac regulation, and is known to be relevant to stress and anxiety assessment [8]. ECG-based stress measures have been shown to correlate with self-reported stress levels.

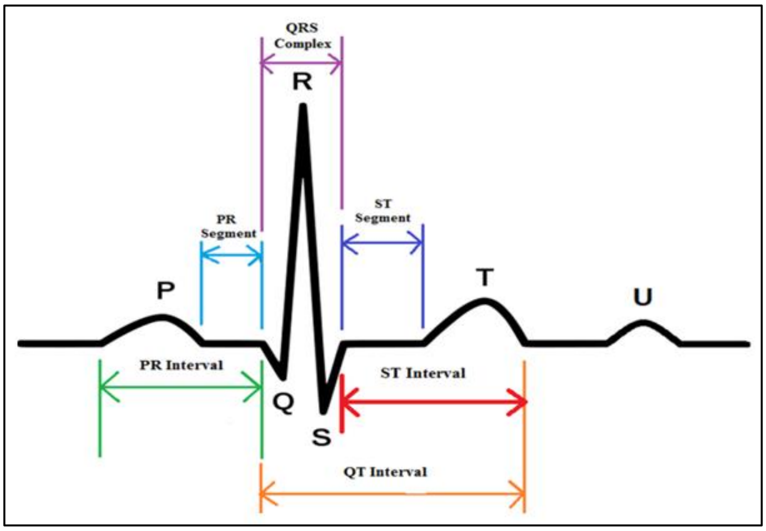

Figure 2.4 ECG waveform features

Figure 2.4 shows an ECG waveform features. The correlation between ECG rhythmic signals and HRV is crucial in understanding the ANS's influence on heart rate fluctuations. HRV, which measures the variation in time intervals between consecutive heartbeats, is derived from the ECG signal and reflects the balance between the sympathetic and parasympathetic branches of the ANS. The ECG rhythmic signals, represented by the electrical activity of the heart, provide the basis for calculating HRV parameters [24]. Changes in the ECG signal, such as the R-R intervals, are indicative of the heart's response to autonomic influences. By analyzing the beat-to-beat variations in the ECG signal, researchers can derive HRV indices that offer insights into the autonomic regulation of heart rate.The correlation between ECG rhythmic signals and HRV is essential for assessing autonomic function, cardiovascular health, and the body's response to stress and other physiological and psychological factors. Understanding this relationship helps in utilizing HRV as a valuable tool in diagnosing clinical conditions and monitoring overall well-being.

2.3.2 Anxiety And Stress Features Based On ECG Waveform

Acute mental stress and anxiety can lead to several notable changes in ECG waveforms, such as increased heart rate, decreased PR and QT intervals, and prolonged QTc interval. Although increased QRS duration and changes in the QRS axis are observed, these are not statistically significant. The precise mechanisms behind these ECG changes remain unclear, but increased sympathetic activity during acute stress is believed to play a significant role. Additionally, certain anxiety medications, like tricyclic antidepressants and SSRIs, may also cause QT prolongation [25].

In patients without known anxiety disorders, temporary nervousness during ECG testing can disrupt readings. For instance, one case study documented a healthy patient who developed quadrigeminy in all ECG leads due to the fear of being shocked by the electrodes, which resolved once the patient was reassured The mechanisms behind these ECG changes are not fully understood, but increased sympathetic activity during acute stress is thought to be a major factor [26]. Despite these stress-induced changes, anxiety alone is unlikely to cause serious ECG abnormalities such as T-wave inversions or QT prolongation. Therefore, healthcare providers should thoroughly consider a patient's complete medical history, including mental health aspects, when interpreting ECG results. It is crucial to distinguish temporary, stress-induced changes from potential underlying cardiac issues to ensure accurate diagnosis and appropriate treatment .

2.3.3 ECG data

Bipolar lead configuration is superior to unipolar configuration for detecting stress and anxiety features in ECG data due to several advantages. Firstly, bipolar leads are more sensitive to changes in the heart's electrical activity, improving the accuracy of stress and anxiety detection. They also reduce noise and artifacts, resulting in a cleaner, more reliable signal. Additionally, bipolar leads offer better spatial resolution, enabling precise localization of heart activity and identification of areas affected by stress and anxiety.

Studies have demonstrated that bipolar leads are more accurate than unipolar leads in capturing detailed heart electrical activity. They better represent heart rate variability (HRV), an important stress and anxiety indicator, and are more effective at detecting arrhythmias associated with these conditions [27]. The enhanced visualization provided by bipolar leads aids clinicians and researchers in diagnosing and monitoring stress and anxiety-related conditions. In summary, bipolar leads offer increased sensitivity, improved signal-to-noise ratio, enhanced spatial resolution, greater accuracy, better HRV representation, improved arrhythmia detection, and superior visualization, making them ideal for detecting stress and anxiety features in ECG data.

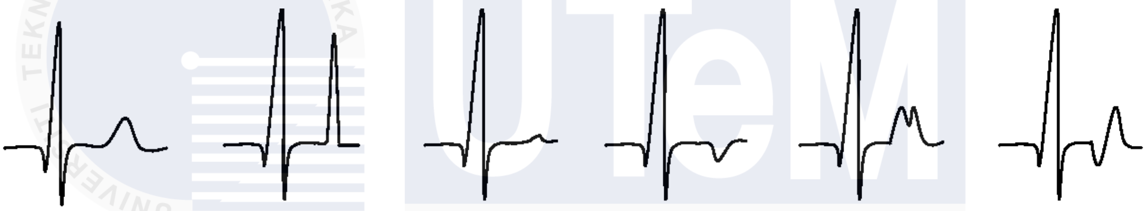

Figure 2.5 Type of ECG waveform

Figure 2.5 shows the possible ECG morphology when monitored using K&H software. For a bipolar configuration of Lead II, it is sufficient to use only three lead clamps. This is because Lead II involves connecting the right arm (RA) to the left leg (LL) and the left arm (LA) to the right leg (RL). This setup allows the ECG module to measure the difference in potential between the right arm and the left leg, and between the left arm and the right leg, which is necessary for the Lead II configuration. Lead II bipolar configuration provides a clear view of the heart's electrical activity that is sensitive to stress and anxiety, requires fewer electrodes, and has a high signal quality, making it ideal for experiments focused on detecting stress and anxiety using ECG biomarkers.

During a relaxed state, the PR interval tends to be longer due to the dominance of the parasympathetic branch of the autonomic nervous system. This branch slows down the heart rate, resulting in a longer PR interval. In contrast, during acute mental stress, the PR interval tends to decrease as the sympathetic nervous system is activated, causing an increase in heart rate and a decrease in the PR interval. This decrease in PR interval is a sign of increased sympathetic activity and a response to stress.Similarly, the QT interval exhibits changes during relaxation and stress. In a relaxed state, the QT interval tends to be longer due to the dominance of the parasympathetic branch, which slows down the heart rate and increases the QT interval. However, during acute mental stress, the QT interval tends to decrease as the sympathetic nervous system is activated, causing an increase in heart rate and a decrease in the QT interval. This decrease in QT interval is also a sign of increased sympathetic activity and a response to stress

During a stressful event, the PR interval tends to decrease. This decrease is a sign of increased sympathetic activity and a response to stress. The parasympathetic branch of the autonomic nervous system, which slows down the heart rate and increases the PR interval, is less dominant during stress. As a result, the PR interval shortens due to the increased heart rate and the decreased parasympathetic influence.

The QT interval also exhibits changes during stress. During acute mental stress, the QT interval tends to prolong due to increased sympathetic activity and decreased parasympathetic activity. This prolongation is a sign of increased sympathetic activity and a response to stress. The QT interval is also influenced by heart rate, and the QT interval shortens with increased heart rate. However, during stress, the QT interval prolongation is more pronounced due to the increased sympathetic activity and decreased parasympathetic activity.

2.4 Past Research

The analysis of the ECG signal has been the subject of extensive research, and various machine-learning algorithms have been developed for ECG-based heartbeat classification [21]. These algorithms can classify different types of heartbeats, such as normal, atrial fibrillation, and ventricular tachycardia, based on the ECG signal features. The development of these algorithms can improve the accuracy and efficiency of ECG-based diagnosis and monitoring of heart diseases and abnormalities.

When it comes to screening and diagnosing cardiac problems, ECGs are very inexpensive and non-invasive [2]. Large volumes of ECG data are now being captured and saved thanks to the introduction of personal ECG monitors; as a result, quick and effective algorithms are developed to evaluate the data and make diagnoses [28], [29]. Anxiety disorders and ANS dysfunction have been linked in several studies. Thus, a better understanding of the physiological markers' accuracy would aid in the accurate detection of anxiety. The physiological signals can provide several traits related to the physiological processes of anxiety, which emphasizes the necessity of pre-processing the data to eliminate artifacts and noise during collection [8].

Table 2.1 Related Previous Research

AUTHOR	METHOD	FINDINGS	REMARKS
Ahn J, Ku Y, Kim H (2019)	The study developed a wearable system for two-channel EEG and one-channel ECG measurement. It was tested using alpha wave EEG and head-measured ECG compared to standard lead I. EEG and HRV features were used to create a stress classification model.	The simultaneous measurement of EEG and HRV improved the accuracy of stress assessments. The model that employed both EEG and HRV features showed superior performance [30].	Future studies should include a larger participant pool, including females, and involve long-term monitoring to assess chronic stress. The use of dry electrodes instead of wet ones is suggested for improved convenience
Pourmohammadi S, Maleki A (2020)	Combination of Electrocardiogram (ECG) and Electromyogram (EMG) signals for stress detection.	EMG and ECG signals can successfully classify stress levels with 100%, 97.6%, and 96.2% accuracy for two, three, and four levels of stress, respectively. The right trapezius muscle's EMG signal is the most effective in recognizing stress.[31]	The study suggests that combining EMG and ECG signals provides a comprehensive approach for stress detection.
L. Gonzalez-Carabarin, E. A. Castellanos-Alvarado, P. Castro-Garcia, and M. A. Garcia-Ramirez (2021)	The study was conducted in a controlled experiment with seven stress-inducing tasks: a questionnaire, relaxation period, timed arithmetic test, unpleasant noise and images, fake interview, timed memory test, and timed logical test. Participants' ECG and EEG signals were recorded throughout.	The study found individual variations in ECG and EEG stress responses, with ECG showing heart rate variability changes and EEG exhibiting frequency band changes. This highlights the need to consider individual differences in stress detection.[32]	The study underscores the importance of using both ECG and EEG biomarkers for accurate stress and anxiety detection. It highlights the need to consider individual physiological differences and calls for further research to understand the ECG-EEG correlation during stress and anxiety.
Ancillon L, Elgendi M, Menon C (2022)	The study reviewed literature from 2012 to 2022 on machine learning applications to biosignals (EEG, ECG, EDA, RSP) for anxiety detection, including 15 relevant studies from databases like PubMed, IEEE, and Embase.	Combining biosignals improved anxiety detection accuracy to 98% in some studies. Effective methods included random forest, support vector machines, and neural networks.[33]	The study highlights machine learning's potential in anxiety detection using biosignals, calls for optimizing methods, and stresses better demographic reporting for generalizable results.

Table 2.1 shows various studies related to wearable systems for stress and anxiety detection using several physiological parameter. Key findings include the effectiveness of combined physiological measurements for improved accuracy, significant individual variations in stress responses, and the potential of machine learning methods for enhancing detection. Future research should focus on diverse participant pools, long-term monitoring, and the use of dry electrodes for better convenience.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the approach and techniques employed in the development of physiological biomarkers through ECG activity analysis for stress and anxiety. This chapter provides an overview of the project flowchart, block diagram, the tools and equipment that are used to fetch ECG data, ECG data collection procedure, and also processing and analysing ECG data.

3.2 Project Overview

This project focuses on developing physiological biomarkers for stress and anxiety through the analysis of ECG data. ECG data will be collected in a controlled environment to ensure consistency and reliability. Subjects will undergo both resting and stress-inducing sessions, during which continuous ECG recordings will be made. Standardized stress-inducing protocols, such as SCWT or mental arithmetic tasks will be employed to induce stress and anxiety responses.

This project also aim to capture a comprehensive range of ECG responses by recruiting a group of subjects and utilizing standardized stress-induction protocols. Advanced signal processing techniques using MATLAB will be applied to extract relevant features from the ECG signals, which will then be analyzed to identify reliable biomarkers. The validity and reliability of these biomarkers will be rigorously tested through cross-validation and independent dataset testing. All procedures involving human subjects will adhere to ethical guidelines, including obtaining informed consent and ensuring data confidentiality.

3.3 Project Block Diagram

The project aims to analyze physiological signals, likely related to heart activity, in response to cognitive tasks. The process begins with data acquisition where participants are presented with stimuli such as mental arithmetic problems or the Stroop Color Word Test, while their physiological signals are recorded.

Next, pre-processing steps like data segmentation and data normalization are applied to increase the quality of data. Feature extraction focuses on identifying key components within the signals, such as QRT peaks.

Moreover, signal processing techniques such as QTc calculation using Bazzett's formula and baseline methods are used to have a more detailed analysis.

Lastly, statistical analysis involves calculating measures like mean, variance, and standard deviation to quantify signal characteristics. Figure 3.1 shows the block diagram of the whole project.

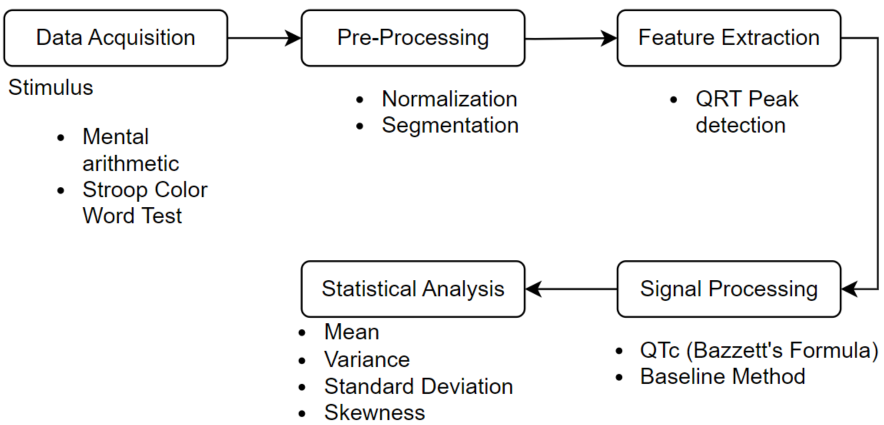

Figure 3.1 Project Block Diagram

3.4 Experimental setup

The experimental setup for this study involves the participation of 10 subjects as the study subject for this project and they are attached with the bipolar lead configuration with KL75001 ECG module to collect ECG data from subjects. The KL75001 ECG module is a non-invasive, wearable device that records the electrical activity of the heart, providing a reliable and accurate measure of ECG signals. The module is connected to a computer, which stores the ECG data in Microsoft Excel files.To induce stress and anxiety in subjects, the Stroop Color Word Test (CWT) is used. The CWT is a widely used protocol that involves presenting a series of words with conflicting colors, which can cause cognitive overload and induce stress. The test is designed to create a conflict between the color of the word and the meaning of the word, requiring subjects to focus on the color of the ink rather than the meaning of the word.The ECG data collected during the CWT is then analyzed using MATLAB. MATLAB is a powerful software tool that provides a range of functions and tools for data analysis, including signal processing, statistical analysis, and visualization. The software is used to extract relevant features from the ECG data, such as heart rate variability, QT interval, and ST segment changes, which are then used to develop physiological biomarkers for stress and anxiety.

3.4.1 K&H Software

To use the K&H software with the KL-75001 ECG Module, start by connecting the KL-75001 ECG Module to the KL-72001 Main Unit. This integration allows for seamless data acquisition and analysis. Next, configure the KL-75001 ECG Module by inserting bridging plugs in positions 1, 2, 3, 4, 5, and 6 to set the high-pass filter (HPF) cutoff frequency to 1Hz, and in positions 9, 10, 11, and 12 or 13 to set the band-rejection filter (BRF) center frequency to 50 or 60Hz, depending on the local line frequency.

Connect the ECG Simulator to the lead side of the KL-79101 5-Conductor Electrode Cable, and the module side to the J1 connector on the KL-75001 ECG Module. This setup allows for the generation of standard ECG signals. Power on the system and select the KL-75001 ECG Module from the LCD display on the KL-72001 Main Unit to ensure that the system is configured for ECG data acquisition and analysis.

Set the MODE SELECT switch to the desired position (e.g., Lead I) to record the ECG waveform. Adjust the VOLT/DIV and TIME/DIV knobs to ensure accurate signal reading. Use the KL-720 program to acquire the measured data via the RS-232 port and display the waveform on the KL-75001 ECG waveform window. Adjust the settings as needed to optimize signal quality and accuracy.

Repeat the process for different leads (e.g., Lead II, Lead III, aVR, aVL, and aVF) by switching the MODE SELECT switch to the corresponding position and recording the waveform. Once the data has been acquired, save the recorded data and exit the KL-720 program. This completes the process of using the K&H software with the KL-75001 ECG Module for ECG data acquisition and analysis. Figure 3.2 shows the graphical user interface of the K&H Software that are used for data acquisition.

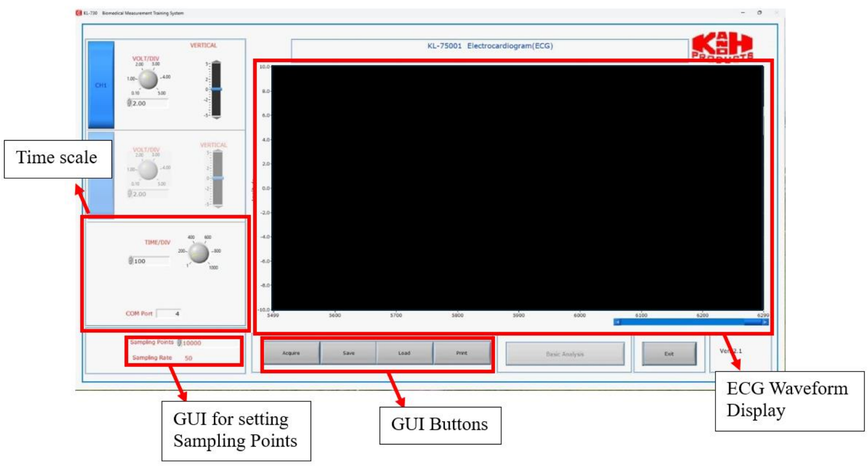

Figure 3.2 GUI of K&H Software

3.4.2 Bridge Connection For KL75001 ECG Module

To connect the bridging plugs on the KL-75001 ECG Module, follow these steps. First, insert bridging plugs in positions 1, 2, 3, 4, 5, and 6 to set the high-pass filter (HPF) cutoff frequency to 1Hz. Next, insert bridging plugs in positions 9, 10, 11, and 12 or 13 to set the band-rejection filter (BRF) center frequency to 50 or 60Hz, depending on the local line frequency.If you need to change the HPF cutoff frequency from 1Hz to 0.1Hz, remove the bridging plugs from positions 5 and 6 and insert them into positions 7 and 8 instead. The bridging plugs are used to configure the HPF cutoff frequency and the BRF center frequency on the ECG module. By changing the positions of the bridging plugs, you can experiment with different filter settings while recording ECG signals. Figure 3.3 shows the bridge connections that must be done before conducting the data acquisition. The figure also highlight the cable connection to computer and also to the ECG sensor. One section of the module are used as the bandpass filtering to filter out unwanted noise from the data acquisition process before pre-processing.

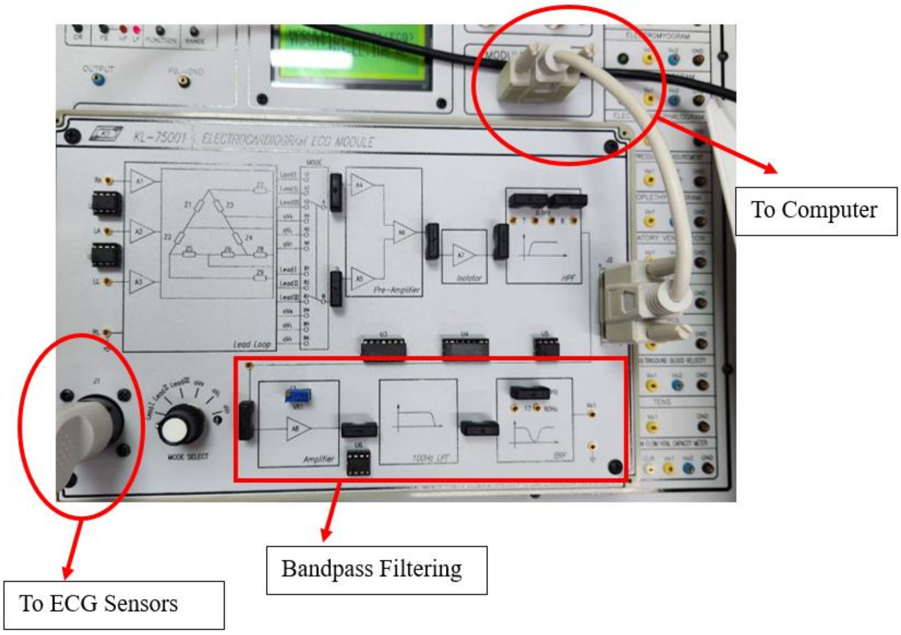

Figure 3.3 Bridge Connections of KL75001 ECG Module

3.4.3 Subject Preparations

For a bipolar Lead II configuration using the KL-75001 ECG Module, the subject needs to be connected with three lead clamps. The right arm (RA) lead clamp is connected to position 1 on the 5-conductor electrode cable, while the left arm (LA) lead clamp is connected to position 2. The left leg (LL) lead clamp is connected to position 3 on the 5-conductor electrode cable. The right leg (RL) is not used in the bipolar Lead II configuration and is left unconnected. This setup allows the ECG module to measure the potential difference between the right arm and left leg, and between the left arm and right leg, which is necessary for the Lead II configuration.

Figure 3.4 Subject Preparation for Data Acquistion

Figure 3.4 shows the subjects preparations before conducting the experiment. Lead clamp placement are placed on both legs and arms. This project is registered under National Medical Research Register (NMRR) as shown in Appendix A. The NMRR is also linked to the review and approval process by the Medical Research and Ethics Committee (MREC). Researchers can submit their proposals for ethical review through the NMRR, ensuring that their research adheres to the highest ethical standards. Additionally, the NMRR serves as a platform for researchers to apply for MOH research grants, with the registration number obtained from the NMRR being required for grant applications.

3.5 Data Acquisition

Before the process of acquiring ECG data from subjects are done, some aspect have to be done first. The first thing to do is to determine the stimulus. Then we, deternmine the configuration of lead which is the type of configuration and the number of lead clamps. A total of 10 subjects have been selected for the ECG data acquisition in which all the subjects are all males.

3.5.1 Data Acquisition Flowchart

This project aim is to develop physiological biomarkers through ECG activity analysis for stress and anxiety. This project is going to utilise K75001 ECG module and MATLAB application. This project also used Microseoft Excel as a storage for ECG data and can be used in data analysing.

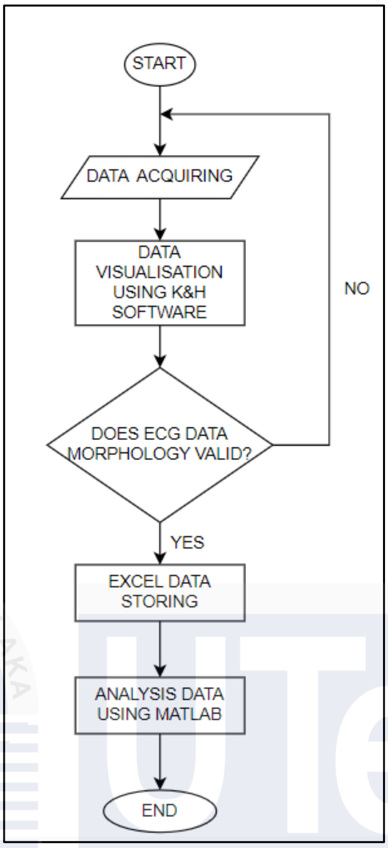

Figure 3.5 Data Acquisition Flowchart

Figure 3.5 shows the project's flowchart. The flowchart outlines a process for detecting stress and anxiety using ECG and EEG data. It starts with acquiring ECG data using monitoring devices to capture heart activity. The data is visualized with K&H software to ensure quality.

Next, the validity of the ECG morphology is checked. If valid, the process continues; if not, the data may need to be re-acquired or preprocessed. Valid ECG data is stored in an Excel file for organization.

The stored data is then analyzed using MATLAB to identify patterns indicating stress and anxiety. Finally, the analyzed ECG data is correlated with EEG data, which measures brain activity. This correlation provides insights into physiological responses to stress and anxiety, aiding in the development of effective mental health diagnostic tools.

3.5.2 Stimulus to induce stress and anxiety

The Stroop Color Word Test (CWT) is a widely used protocol to induce cognitive stress and reliably activates the sympathetic nervous system (SNS). It involves presenting a series of words with conflicting colors, which can cause cognitive overload and induce stress and anxiety in subjects [34].

This conflict can lead to increased physiological and psychological responses, including increased heart rate, blood pressure, and skin conductance, as well as feelings of anxiety and frustration.

The CWT is considered a reliable method for inducing stress and anxiety in subjects because it is easy to administer and can be standardized to ensure consistent results. It has been used in various studies to investigate the effects of stress on physiological and psychological responses [35].

Figure 3.6 SCWT (Left) & Mental Arithmetic (Right)

Firgure 3.6 shows an example of SCWT game on the left and mental arithmetic game on the right. Both game are designed to evoke stress and anxiety by creating a sense of cognitive dissonance. SCWT requires subjects to focus on the color of the ink while simultaneously processing the meaning of the word. This dual-task processing can lead to increased cognitive load, which can cause feelings of frustration, anxiety, and stress. Additionally, the test's design can create a sense of uncertainty and unpredictability, as subjects are unsure of the correct answer and must make quick decisions under time pressure. These factors combined can induce a significant amount of stress and anxiety in subjects, making the SCWT a useful tool for studying the effects of stress on cognitive and physiological processes.

3.5.3 Procedure

Upon arrival, each subject was connected to the KL75001 ECG module to begin recording their ECG data. The subjects were instructed to sit comfortably in a room and were allowed 10-15 minutes to acclimate to the environment and equipment. After the subject were acclimited with the environment, baseline ECG data was recorded while the subjects were at ease for 5 minutes. After the initial data recording session is done, subjects were given a 5-minute rest break to stretch and relax.

They were then informed that they would be completing a test that are designed to induce anxiety and stress. The subjects were given detailed instructions on the test and were allowed to ask any clarifying questions before beginning.The anxiety and stress-inducing test consisted of a series of challenging cognitive tasks and social evaluative components. Subjects were asked take a SCWT test and also mental arithmetic in a game form. The test lasted for 15 minutes, and ECG data was recorded continuously throughout the duration of the test.

All ECG data collected during the baseline and stress-inducing test periods was stored for subsequent analysis. The data was labeled with unique subject identifiers to protect confidentiality and ensure accurate matching of baseline and stress-induced ECG recordings for each subject. Figure 3.7 shows subject during a data acquisition session for stress test.

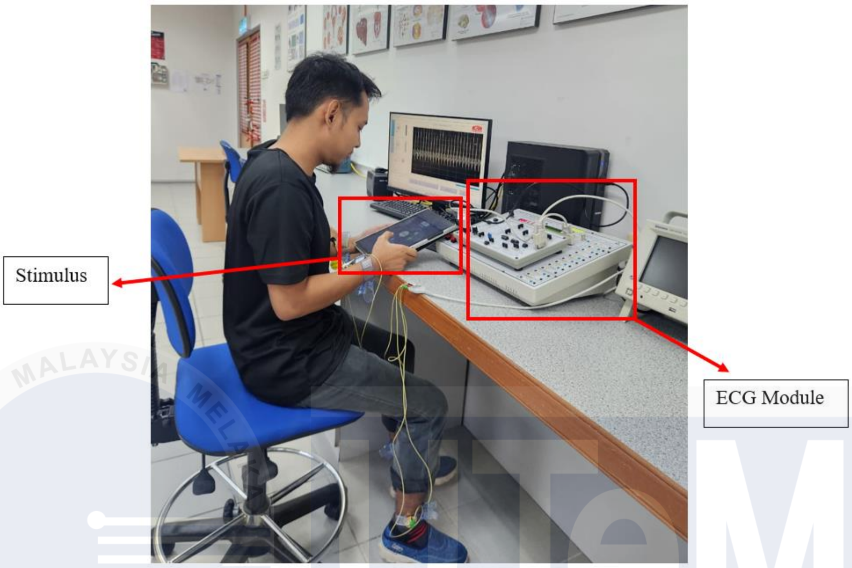

Figure 3.7 Subject During Stress Test

3.6 ECG Data Pre-processing

To get a precise ECG data analysis, some preprocessing steps are needed to be used. This includes data segmentation and data normalization.

3.6.1 Data Segmentation

In ECG data processing, data segmentation plays a critical role by dividing the continuous ECG signal into smaller, manageable segments or windows based on specific criteria, such as time duration or cardiac events like the PQRST complex. Additionally, segmentation improves analysis accuracy by enabling calculations like mean, variance, and skewness to be more localized, reflecting specific conditions such as stress or non-stress episodes. It also aids in detecting abnormalities, like arrhythmias, by focusing on specific cardiac events and variations within defined windows. Furthermore, segmentation prepares the data for machine learning or pattern recognition algorithms, which often require fixed-length or event-specific inputs for accurate trend identification and classification.

3.6.2 Normalization of Data

Normalizing data ensures uniformity across the ECG dataset by scaling signals to a common range or standard format. This process minimizes variability introduced by differences in recording devices, electrode placement, or other external factors, ensuring that all signals are comparable.

$$Normalize\ signal = \frac{x - \min(x)}{(\max(x) - \min(x))} \times 2 - 1 \qquad \text{---------------} \quad \textbf{Eq 3.1}$$

It also improves the performance of machine learning models by allowing algorithms to process features equally, preventing large amplitudes from dominating the training process and enabling faster convergence. Normalization highlights subtle changes or patterns in the ECG signal, such as slight variations in QT intervals during stress, and facilitates direct comparisons between patients by bringing all data to a common scale. Together, data segmentation and normalization form the foundation of reliable ECG data analysis, enabling accurate detection of cardiac patterns and anomalies.

3.7 Feature Extraction

The process of finding and separating important patterns or traits from unprocessed data in order to make analysis and interpretation easier is known as feature extraction. It includes recognizing important characteristics of ECG signals, including the QRT peak.

3.7.1 QRT Peak Detection

It is important to detect all the ECG morphology peak as the peak provides valuable information for further analysis.

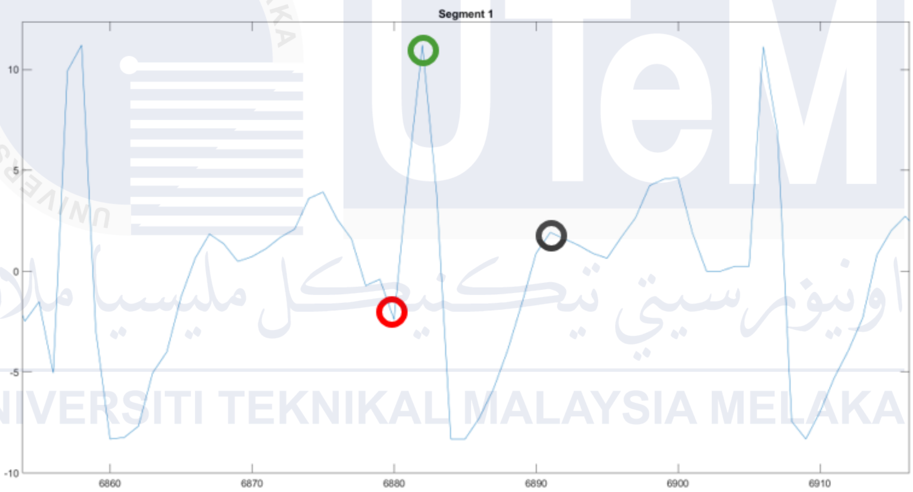

Figure 3.8 ECG Morphology

Figure 3.8 shows the ECG morphology with detected peaks. Red circle is Q peak, green circle is R peak, and black circle is T peak. All the detected peakswill be used for further processing and analysis to get a good understanding of what was the ECG activity was like.

The duration between the beginning of ventricular depolarization (Q-wave) and the ending of repolarization (T-wave) is measured by the QT interval. It is used to assess cardiac disorders, identify extended QT syndromes, and monitor the effects of medications on the heart.

- QT interval

$$QT = t_T - t_Q \qquad \text{---------------} \quad \textbf{Eq 3.2}$$

$t_T = time\ of\ the\ end\ of\ T\ wave,$
$t_Q = time\ of\ the\ start\ of\ the\ Q\ wave$

One of the most important metrics for heart rate and variability study is the RR interval, which is the period of time between consecutive R-peaks. It is essential for determining stress levels, identifying arrhythmias, and analyzing autonomic nervous system activity.

- RR interval

$$RR = t_{R2} - t_{R1}$$

t_{R2} & $t_{R1} = times\ of\ two(2)\ successive\ R\ peaks$ --------------- **Eq 3.3**

3.8 Signal Processing

For the signal processing part, two methods are used to get and accurate data for statistical analysis. The methods are finding corrected QT (QTc) that are extracted using Bazzett's formula in MATLAB and the second method are baseline method that uses a baseline as threshold for stress detection depending on the which gender that the ECG data are drawn out of.

3.8.1 Corrected QT (QTc) using Bazzett's formula

The QT interval is frequently adjusted for changes in heart rate using Bazett's method. The formula uses the RR interval to convert the QT interval (measured in seconds) to a normalized heart rate. This adjustment makes sure that different heart rates do not impact QT interval comparisons, which makes it useful for evaluating the effects of medications or cardiac health.

$$QTc = \frac{QT}{\sqrt{RR}} \qquad \text{---------------} \quad \textbf{Eq 3.4}$$

3.8.2 Baseline Method

Because of physiological differences, men and women have different baseline thresholds for QTc. A QTc interval is considered prolonged for males if it is longer than 450 ms, and for women if it is longer than 460 ms. Men's and women's borderline values are 431–450 ms and 451–460 ms, respectively, with both having a crucial prolongation over 500 ms [36].

Table 3.1 QTc Baseline based on gender

Gender	Stress QTc baseline
Male	≥450
Female	≥460

3.9 Statistical Analysis

The analysis of ECG data using heart rate (HR) vs. condition and statistical methods like standard deviation, variance, skewness, and kurtosis provides both macro- and micro-level insights into cardiac behavior. HR vs. condition reveals overall physiological responses to stress, such as increased HR under stress due to autonomic activation. Together, these methods provide a comprehensive understanding of how stress impacts cardiac activity, balancing simplicity with detailed insights for robust health monitoring and stress detection.

3.9.1 HR vs Condition

In ECG analysis, determining a dataset's quality guarantees the dependability and efficiency of algorithms or models in identifying characteristics, categorizing ailments, and assisting with diagnosis. It determines how well the outcomes match the ground reality, which aids in validating the analysis's effectiveness, reducing mistakes, and guaranteeing accurate clinical judgment.

$$Accuracy = \frac{correct\ sample}{total\ sample} \times 100\% \qquad \text{---------------} \quad \textbf{Eq 3.5}$$

A normal heart beat during non stress period are approximately 60 until 100 beat per minute while for the stress period the heart beat tends to be increased to above 100 beat per minute.

$$\text{Heart Rate (bpm)} = \frac{60}{\text{RR}} \qquad \text{---------------} \quad \textbf{Eq 3.6}$$

Condition	Range
Normal	60 - 100
Stress	>100

Figure 3.9 BPM Condition vs Range

Figure 3.9 shows the BPM in range for different conditions. It is observable that during stress state, the range of the BPM readings will increase due to increased autonomic nervous system activity.

3.9.2 Statistical Analysis

- Mean

$$\mu = \frac{1}{N}\sum_{i=1}^{N} x_i$$

$\mu = Mean\ dataset$ ---------------- **Eq 3.7**

$N = Total\ number\ of\ data\ points$

$x_i = Individual\ data\ points$

The mean represents the average value of a specific ECG feature, such as the QT interval, and provides the central tendency of the dataset. In an ECG dataset, the mean value helps identify whether the feature falls within a normal range. For example, a normal QT interval mean typically ranges between 350–450 ms, depending on heart rate and demographics. Deviations from the normal range can indicate potential cardiac abnormalities. A prolonged QT mean may suggest arrhythmias or other heart-related issues, while a shortened QT mean could indicate electrolyte imbalances or genetic conditions. In the context of stress, changes in the mean QT interval may reflect the heart's response to physiological or psychological strain, highlighting its significance in detecting abnormal cardiac behavior.

- Variance

$$\sigma^2 = \frac{1}{N}\sum_{i=1}^{N} (x_i - \mu)^2$$ ---------------- **Eq 3.8**

Variance measures how spread out the ECG data points are around the mean and reflects the overall variability within the dataset. Low variance indicates that most feature values, such as QT intervals, are close to the mean, signifying stable and consistent cardiac activity. On the other hand, high variance suggests greater variability, which could indicate irregularities in heart rhythms or autonomic nervous system responses, as observed during stress or illness. In an ECG dataset, variance helps identify differences in cardiac patterns under varying conditions. For example, stress conditions often increase variance, reflecting physiological variability and irregular repolarization patterns. This measure is critical for distinguishing between normal and abnormal heart activity.

- Standard deviation

$$\sigma = \sqrt{\sigma^2} \qquad \text{---------------} \quad \textbf{Eq 3.9}$$

Standard deviation is the square root of variance and quantifies the average deviation of ECG feature values from the mean in the same units (e.g., milliseconds for QT intervals). It provides a more intuitive understanding of data variability. A low standard deviation in an ECG dataset indicates consistent and regular cardiac activity, while a high standard deviation reflects greater variability, which may signal irregularities. For instance, stress-induced conditions often result in a higher standard deviation in QT intervals, indicating increased cardiac variability and potential instability due to autonomic nervous system activation. Conversely, in non-stress conditions, a lower standard deviation represents stable and regular cardiac activity, making it a reliable indicator of physiological stability.

- Skewness

$$g_1 = \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^3}{\left(\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2\right)^3} \qquad \text{---------------} \quad \textbf{Eq 3.10}$$

Skewness measures the asymmetry of the data distribution in an ECG dataset, providing insights into how QT intervals or other cardiac features are distributed around the mean. A positive skewness indicates a distribution with a longer tail toward higher values, which suggests that there are more instances of longer QT intervals. This pattern is often observed under stress conditions, where the variability in cardiac repolarization increases, potentially elevating the risk of arrhythmias. Conversely, a skewness closer to zero or slightly negative implies a more symmetric distribution, typically seen under non-stress conditions, indicating stable cardiac activity. By assessing skewness, researchers can determine how stress or other factors affect the balance of QT interval durations, offering valuable insights into the underlying cardiac behavior.

- Kurtosis

$$g_2 = \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^4}{\left(\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2\right)^4} \quad \text{---------------} \quad \textbf{Eq 3.11}$$

A kurtosis of 3 corresponds to a normal distribution. Higher values (leptokurtic) indicate heavy tails, and lower values (platykurtic) indicate light tails. Kurtosis measures the "tailedness" or sharpness of the peak of the data distribution, highlighting the prevalence of extreme values. A high kurtosis in an ECG dataset indicates a distribution with heavy tails and a sharp peak, suggesting that extreme QT interval values—both very short and very long—are more common. This is often observed under stress conditions, where physiological responses can lead to outlier events, such as arrhythmias. On the other hand, low kurtosis reflects a flatter distribution with fewer extreme values, as seen in non-stress conditions, indicating more consistent and stable QT intervals. The kurtosis metric is essential for identifying how stress impacts the frequency of extreme cardiac events and assessing the risk associated with irregular heart patterns.

3.10 Sustainable Development Goals

The Sustainable Development Goals (SDGs) that are directly related to anxiety, stress, or mental health are primarily SDG 3 which is Good Health and Well-being. This goal includes specific targets for mental health, wellbeing, and substance abuse. For instance, Target 3.4 ,within the SDG 3, aims to reduce premature mortality from non-communicable diseases by one-third through prevention and treatment, while also promoting mental health and well-being. Additionally, Target 3.5 also within the SDG 3, focuses on strengthening the prevention and treatment of substance abuse, including narcotic drug addiction, and supporting policies and programs to prevent the use of controlled substances and to treat and support those recovering from addiction [37].

Mental health is also connected to other SDGs. For example, SDG 1: No Poverty highlights the influence of poverty and social adversity on mental health, and addressing poverty can have a positive impact on mental health outcomes. Similarly, SDG 10: Reduced Inequalities emphasizes that mental health disparities are often exacerbated by social and economic inequalities, and addressing these inequalities can help reduce mental health disparities. Furthermore, SDG 16: Peace, Justice and Strong Institutions recognizes that mental health is affected by conflicts and trauma, and addressing these issues can contribute to improved mental health outcomes.

These connections underscore the importance of addressing mental health as a critical component of sustainable development efforts. By focusing on mental health, we can work towards achieving a more comprehensive and equitable approach to development that prioritizes the well-being of individuals and communities.

3.11 Summary

This chapter outlines the methodology for developing physiological biomarkers for stress and anxiety through ECG activity analysis. The project involves collecting ECG data from subjects in a controlled environment using the KL75001 ECG module. Subjects undergo baseline and stress-inducing sessions, with standardized protocols like the Stroop Color Word Test (SCWT) used to induce stress and anxiety. The ECG data is stored in Microsoft Excel and analyzed using MATLAB to extract features such as heart rate variability, QT interval, and ST segment changes. The bipolar lead configuration is employed for its superior sensitivity, reduced noise, and better spatial resolution, enhancing the accuracy of stress and anxiety detection. Ethical guidelines are strictly followed, including informed consent and data confidentiality. The collected ECG data is preprocessed to remove noise, and advanced signal processing techniques are applied to identify reliable biomarkers. These biomarkers are validated through rigorous cross-validation and independent testing to ensure their reliability and applicability in real-world settings.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

All the results that have been processed and analysed will be presented in this chapter. Starting from the data acquisition process and end at statistical analysis. This chapter will demonstrate the success of this project at developing stress biomarkers through physiological analysis using MATLAB software.

4.2 Raw Data From KL75001 ECg Module

From the data acquisition process, the subject's ECG data will first be displayed using Excel to verify the validity of subject's ECG morphology before pre-processing.

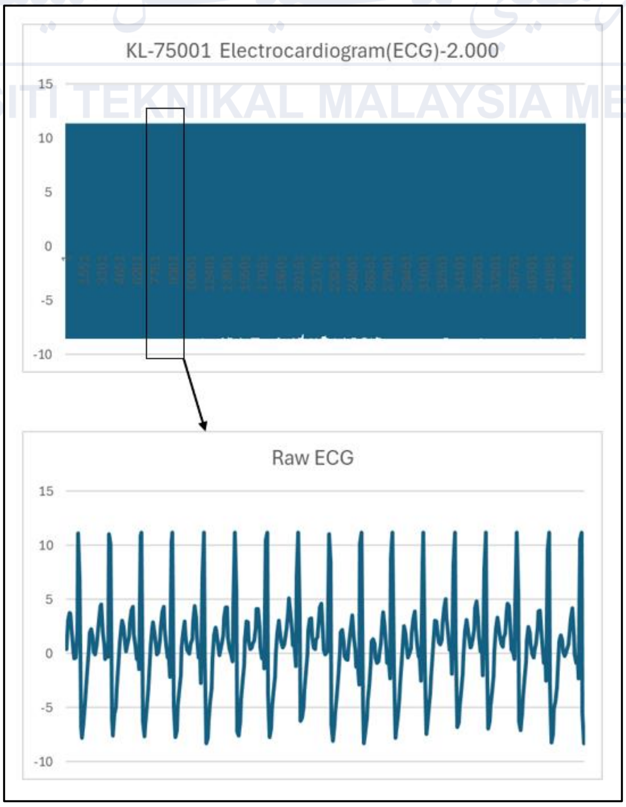

Figure 4.1 Raw ECG data Visualisation in Excel

Figure 4.1 shows the visualization of raw ECG data using Excel. The KL75001 ECG module provided a dataset containing 45,000 data points, representing the electrical activity of the heart over time. This raw dataset is essential for analyzing cardiac function and serves as the foundation for subsequent preprocessing and analysis steps.

4.3 Raw Data Visualisation Using MATLAB

Aside from using Microsoft Excel, this project also used MATLAB software to visualize the ECG morphology that are gathered from the data acquisition process.

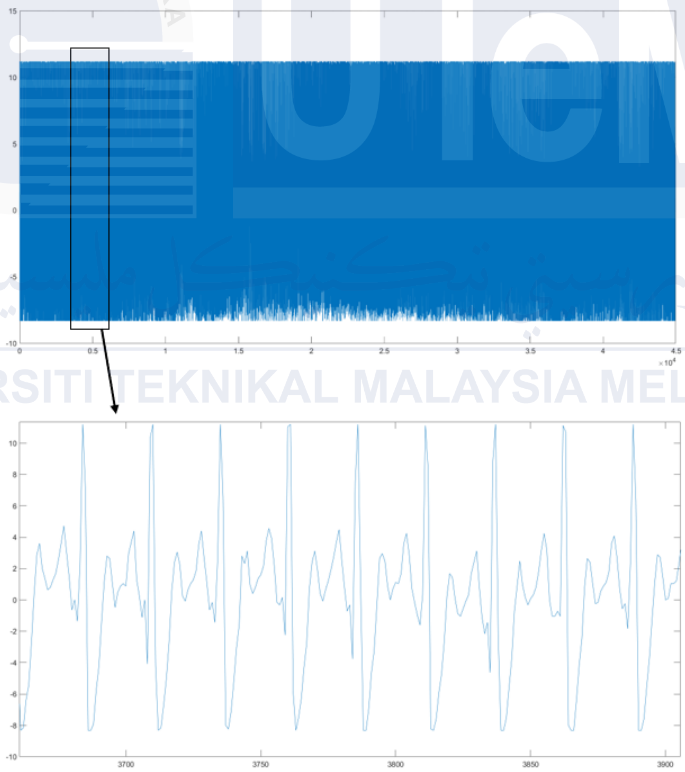

Figure 4.2 Raw ECG Data Visualisation Using MATLAB

Figure 4.2 shows the raw ECG data before preprocessing using MATLAB software. The raw ECG data was visualized using MATLAB, which allowed for the graphical representation of the signal. This visualization helps in identifying patterns, irregularities, and noise within the data. Such insights are crucial for determining preprocessing requirements and ensuring accurate feature extraction.

4.4 Preprocessing Raw ECG Data

Preprocessing involves cleaning and transforming the raw ECG data to make it suitable for analysis.

4.4.1 Data Segmentation

This segmentation facilitates precise feature extraction by isolating key intervals, such as the QT interval or RR interval, ensuring that the most relevant portions of the signal are analyzed while reducing noise and irrelevant data.

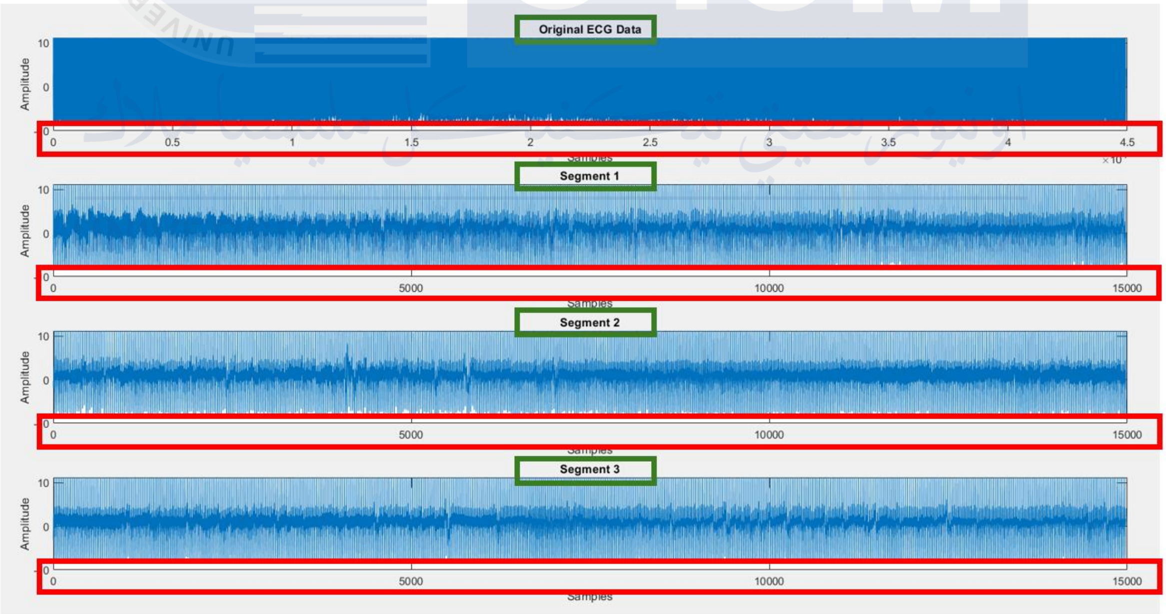

Figure 4.3 One Dataset Segmented Into three Segment

Figure 4.3 shows the segmentation of data from one long data set that contains 45k data points into 3 equal segment of 15k data points each. ECG morphology can be observed better after the data segmentation process.

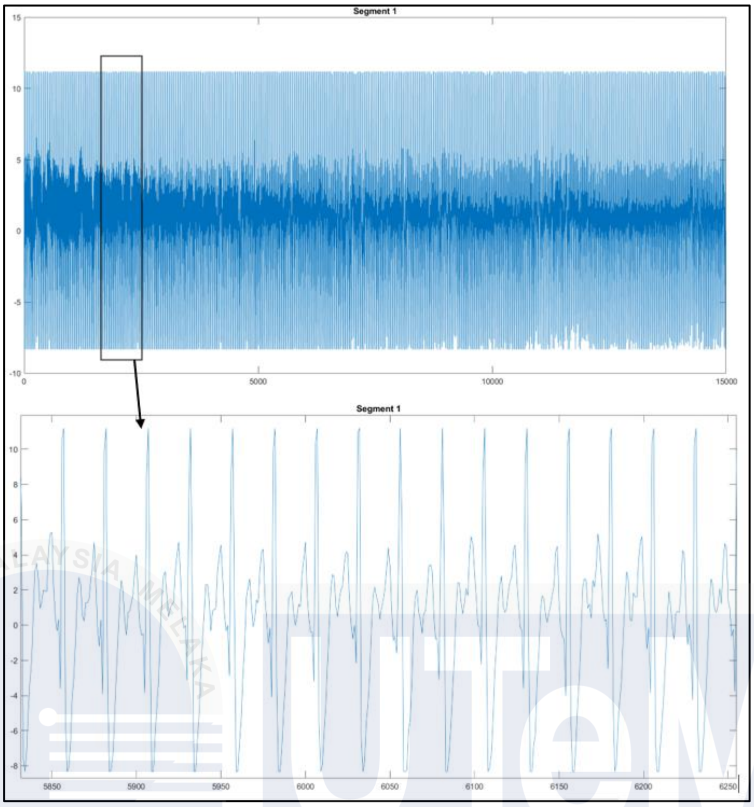

Figure 4.4 Data Segmentation

Figure 4.4 show ECG morphology from a segment of ECG data that was zoomed in. Although the raw data was segmented into smaller parts, with a maximum of 15,000 points per segment, the ECG features' morphology is not clearly visible to ensure consistent amplitude across segments. While segmentation improves processing and analysis efficiency, the amplitude appears consistent with the original data before segmentation. However, this suggests that further segmentation is necessary to achieve a clearer observation of the ECG morphology. Proper segmentation plays a crucial role in visualizing the features of the ECG more distinctly.

4.4.2 Normalizing Data

Normalization enhances the quality of the data for analysis by eliminating biases, ensuring that extracted features reflect physiological conditions rather than technical inconsistencies.

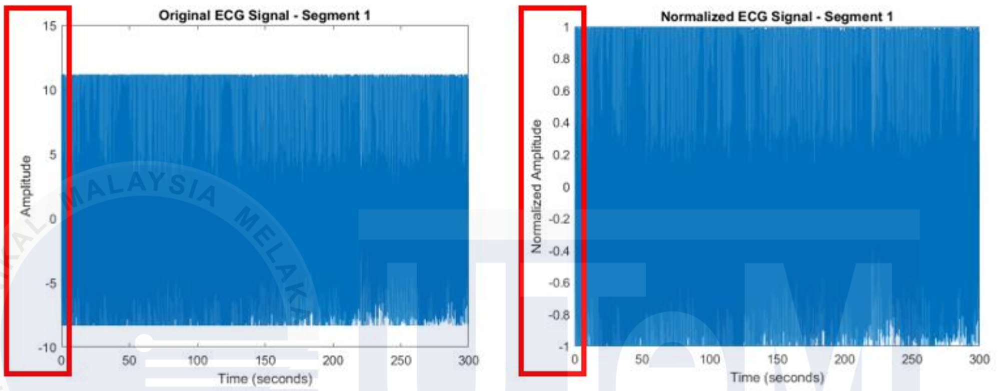

Figure 4.5 Amplitude Normalization

Figure 4.5 shows the data before normalization on the left and data after normalization on the right. It can be observed that there is difference in amplitude after the data was normalized.

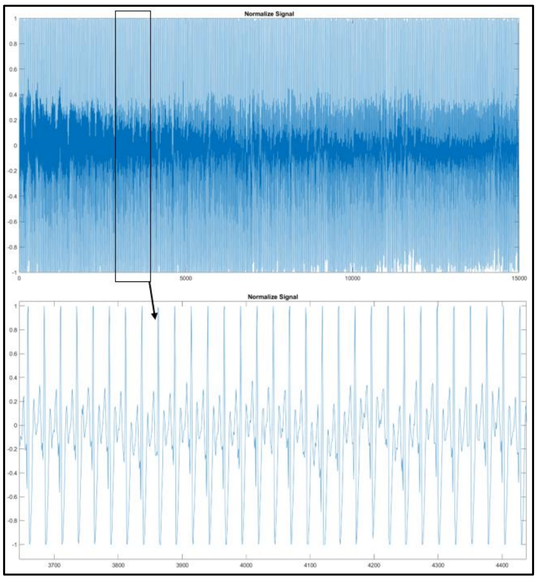

Figure 4.6 Data Normalization

Normalization was applied to adjust the amplitude range from 10 to 1, ensuring uniformity across the dataset. This step minimizes variations caused by external factors such as electrode placement or signal acquisition conditions while keeping the ECG morphology undisturbed.

4.5 Feature Extraction

QT interval and RR interval are able to be measure from the peak detection. These characteristics offer necessary information for accurately identifying cardiac disorders, analyzing heart rate variability, and determining how well the heart is functioning overall.

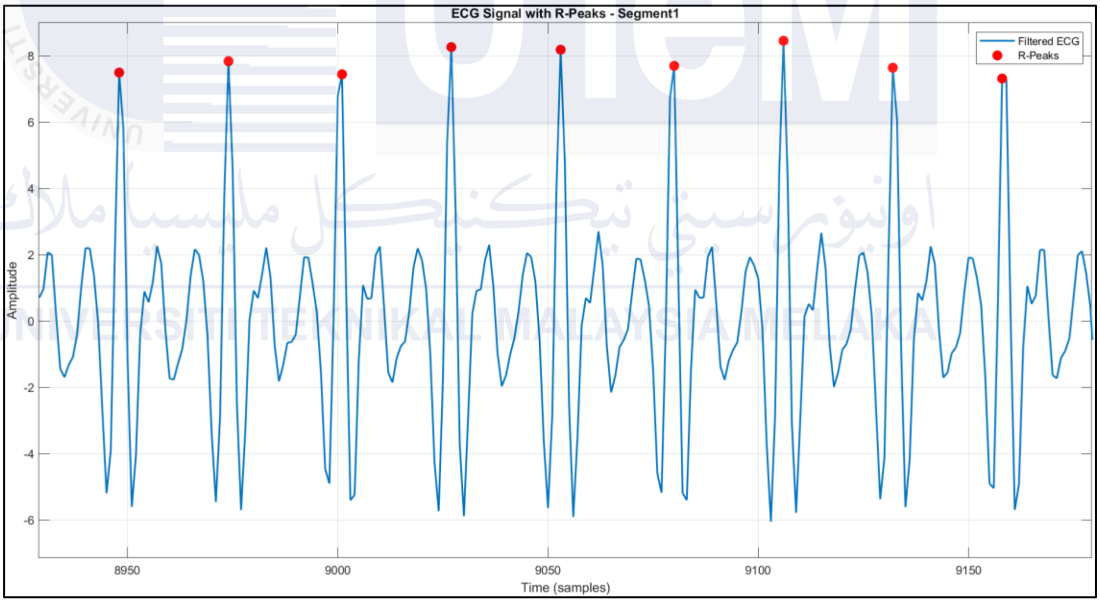

Figure 4.7 R-peak detection

Figure 4.5 shows the detected R-peaks. R-peaks, which correspond to ventricular depolarization (heartbeats), were identified in the ECG signal. Accurate detection of these peaks is critical for calculating heart rate and analyzing heart rate variability (HRV).

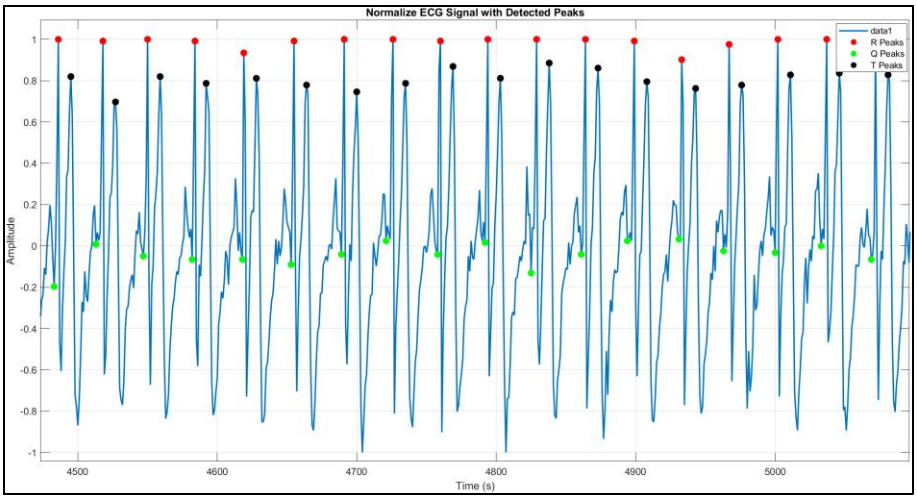

Figure 4.8 QRT-peak Detection

Figure 4.6 shows the detected QRT-peaks. QRT peaks represent specific points in the cardiac cycle (Q-wave, R-wave, T-wave). Detecting these peaks provides information on the timing and morphology of heartbeats, which is essential for assessing cardiac health.

4.6 Signal Processing

From the QRT-peak detection, it is possible to measure the QTc interval using Bazzett's formula in MATLAB software. Next, a baseline of 450ms are used to detects all the QTc interval that exceed the limit as all the subjects are males.

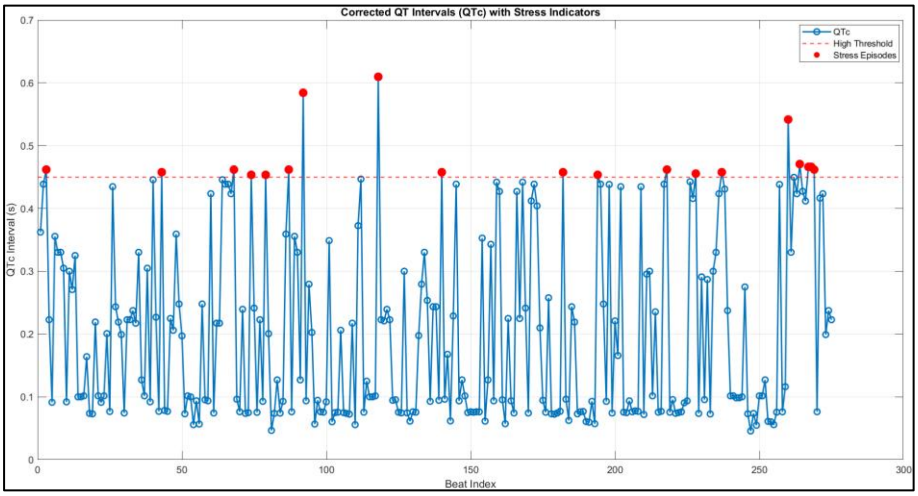

Figure 4.9 QTc During Stress Episode

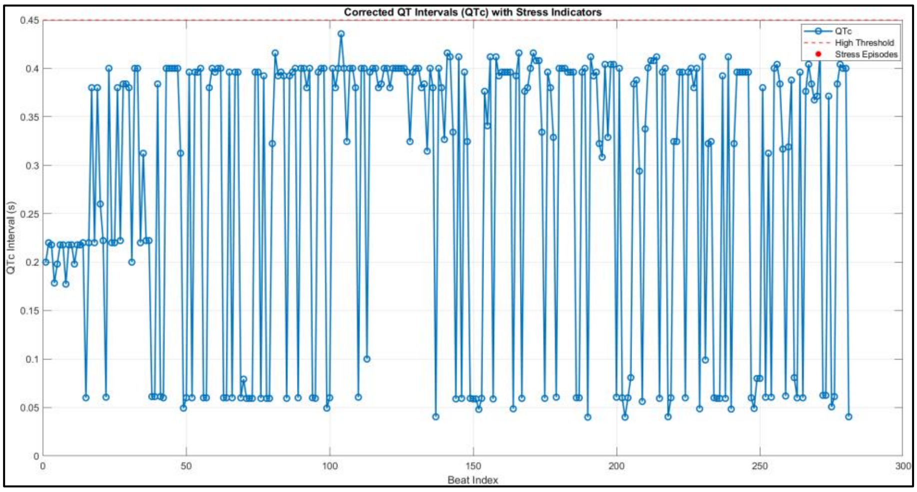

Figure 4.10 QTc During Non Stress Episode

Figure 4.9 and 4.10 shows the plotted QTc intervals. The plot shows QTc intervals extracted from male participants, using a baseline threshold of 0.45 seconds to identify stress episodes. Most QTc values fall below the threshold, indicating normal conditions, while spikes above 0.45 seconds, marked with red dots, signify potential stress episodes. These stress events are sporadically distributed but cluster around beat indices 100–120 and 240–260, with some peaks exceeding 0.5 seconds, suggesting heightened stress or potential cardiovascular risks. The data highlights the importance of using male-specific QTc thresholds and could be used to correlate stress episodes with external triggers or other physiological parameters, offering insights into cardiac stress responses and risk monitoring.

4.7 Analysis

After all the steps before have been completed, analysis is the last steps in this project to get a result. The analysis covers the HR condition and also through statistical measurements.

4.7.1 HR vs Condition

	During Stress	
Segment	HR	BPM
1	425	85.096
2	432	86.498
3	406	81.292
4	511	102.549
5	518	103.718
6	530	106.120
7	520	104.118
8	516	103.317
9	515	103.117
10	510	102.116

Figure 4.11 HR During Stress

Stress	Normal	3
	Stress	7

Figure 4.12 Ratio Between In Range and Abnormal Data

Figure 4.11 and 4.12 shows the data of BPM for 10 segment during stress period. It is observeable that most of the data are above in the range of BPM during stress period. It is possible that some of the data that are below the stress BPM range might be due to untriggered stress response and these segment might have to use different stimuli to trigger their stress response. For the accuracy of the stress period ECG data, this project achieved 70% of accuracy.

$$Accuracy = \frac{7}{10} \times 100\%$$

$$Accuracy = 70\%$$

	During Non Stress	
Segment	Heart Rate	HR (BPM)
11	428	85.892
12	709	101.532
13	381	76.460
14	392	78.667
15	665	95.231
16	678	97.093
17	466	92.283
18	453	90.909
19	467	93.719
20	410	82.280

Figure 4.13 HR During Non Stress

Non stress	In range	9
	Abnormal	1

Figure 4.14 Ratio Between In Range and Abnormal Data

Figure 4.13 and 4.14 shows the data of BPM for 10 segment during normal period. It is observeable that most of the data are in the range of BPM during normal period. There is a possibilities that the one segment that are out or the range of normal BPM indeed in stress period due to external stressor. This project achieved an accuracy of 90% for the normal period.

$$Accuracy = \frac{correct\ sample}{total\ sample} \times 100\%$$

$$Accuracy = 90\%$$

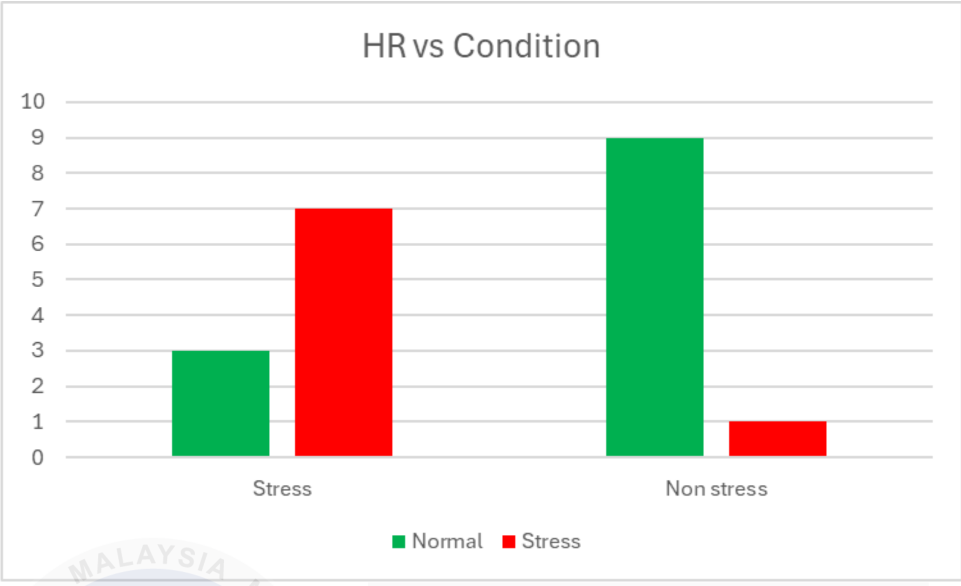

Figure 4.15 HR vs Condition

Figure 4.9 show the data reliability for 20 segments of data. It shows a clear elevation in HR during stressful conditions compared to non-stressful ones, highlighting the body's physiological stress response and the potential impact of chronic stress on cardiovascular health.

4.7.2 Statistical Analysis

Statistical analysis captures detailed variability, asymmetry, and the prevalence of extreme events in ECG features like QT intervals.

		Average Readings				
		Mean QT	Variance QT	Std Dev QT	Skewness QT	Kurtosis QT
Condition	Stress	0.19821	0.01428	0.11613	0.49603	3.17148
	Non Stress	0.22764	0.01251	0.10892	-0.48201	1.67403

Figure 4.16 Average Reading for Statistical Analysis

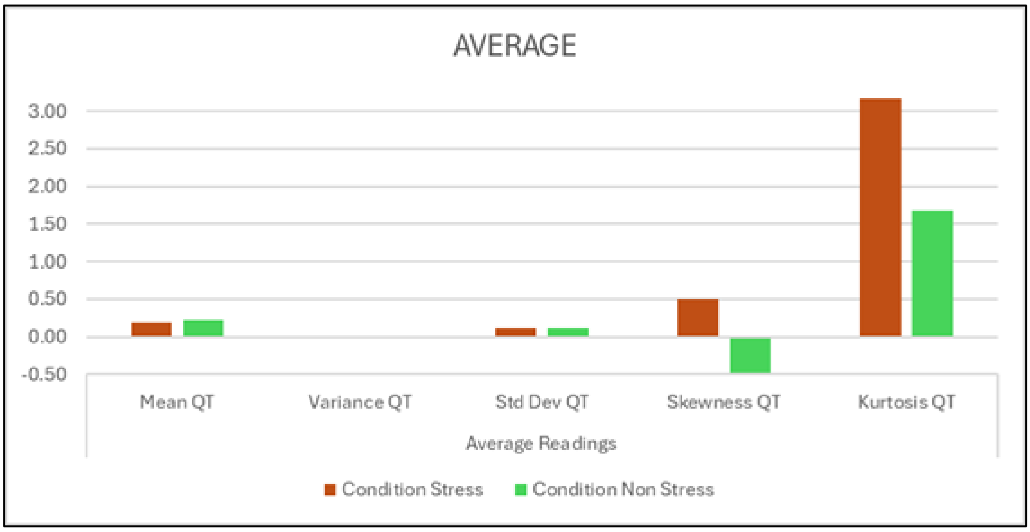

Figure 4.17 Visualisation of Statistical Analysis

Figure 4.16 and 4.17 show the statistical analysis of 20 segments of ECG data. Skewness is a measure of the asymmetry of a distribution. Positive skewness indicates a longer tail on the right, while skewness closer to zero or negative indicates symmetry or a longer tail on the left.

Positive skewness in QT intervals suggests a longer tail on the right side of the distribution, indicating more variability and a tendency toward longer QT intervals and it suggest a data in stress conditions . Stress conditions, such as physical or emotional stress, can lead to increased sympathetic nervous system activity, which may prolong QT intervals due to changes in heart rate and repolarization dynamics. This can result in a distribution of QT intervals that is skewed to the right.

Skewness closer to zero or negative suggests a more symmetric distribution of QT intervals, indicating less variability and a tendency toward shorter or more consistent QT intervals. Under non-stress conditions, the autonomic nervous system is more balanced, leading to stable heart rate and repolarization patterns. This results in a more symmetric distribution of QT intervals.

The kurtosis values further support this variation, with significantly higher kurtosis observed under stress conditions. High kurtosis suggests a distribution with heavy tails and a sharp peak, implying that extreme QT interval values, both long and short, are more frequent under stress. This could signal a greater likelihood of outlier events, such as arrhythmias, during stress. On the other hand, the lower kurtosis under non-stress conditions reflects a flatter distribution with fewer extreme values, indicating more consistent and stable QT intervals.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This project successfully detects stress episodes by analyzing the QTc interval from ECG signals using MATLAB. The project began by identifying ECG signal biomarkers most likely to be influenced by stress. Stress can be triggered by various stimuli, and this project utilized the SCWT and mental arithmetic tasks as controlled stressors. These stimuli effectively elicited physiological stress responses, enabling a detailed analysis of their impact on the heart's electrical activity.

This project further process stress episodes by detecting the QRT peak to calculate the QT and RR interval of the ECG signals. From the QT and RR interval that are identified, Bazett's formula are then employed to calculate the QTc interval. This adjustment accounted for heart rate variability, ensuring accurate detection of stress-induced changes. From Bazett's formula, QTc intervals were extracted and analyzed in detail to quantify stress responses under different conditions. The baseline method established a reliable threshold for distinguishing stress episodes from non-stress conditions based on variations in ECG biomarkers. This provided a systematic and effective framework for identifying stress occurrences.

Lastly, this project used statistical analysis using mean, standard deviation, variance, kurtosis, and skewness. The mean indicates the average QT interval, while standard deviation and variance assess variability, with higher values suggesting irregularities, often linked to stress. Kurtosis reflects the presence of extreme QT values, with higher kurtosis indicating sharper peaks and heavy tails under stress conditions.

Skewness measures asymmetry, where positive skewness suggests a tendency for longer QT intervals, commonly seen during stress. Together, these statistical analysis helps to identify physiological changes and detect stress-induced effects.

In conclusion, this project demonstrated the feasibility of detecting stress episodes using ECG biomarkers, particularly the QTc interval. The integration of controlled stimuli, baseline thresholding methods, and computational tools in MATLAB highlights the potential of this approach for applications in health monitoring, stress management, and personalized interventions aimed at mitigating stress-related health risks.

5.2 Future work

Throughout the completion of this project, a few aspects can be improved to make this project to run more smoothly and the accuracy of the data can be increased and the improvement can be implemented in the future.

5.2.1 Data Acquisition

As for the data acquisition part, two activities are done as the stimulus in triggering the stress response in subject. The activities are mental arithmetic and SCWT. Other activities that are proven at triggering stress response such as public speaking could also be implemented in the future.

In terms of choosing subject for the procedure, a subject can be and possibly should be picked from various group, background, and gender to get more data variety. The idea of a variety in subject groups is to ensure the stimulus that are given to subjects are effective in triggering stress.

5.2.2 Stress Detection

As for this project, the use of anova and HRV are being used as stress biomarkers and it is recommended that in the future the use of RR interval and other features are implemented to develop stress biomarkers. The use of two or more features are recommended to develop a biomarker to visibly observe the correlation between ECG features at detecting stress and increase the accuracy of a dataset from the data acquisition.

5.2.3 Future Development

Integrating this project with artificial intelligence(AI) for future development seems to be a wise thing to do. AI are able to process more complex physiological biomarker data with improve accuracy with machine learning. The ability of machine learning to learn something new with every sample that are provided for it, such as HRV and cortisol levels, is going to help us detect sign of stress making AI an efficient tool for detecting stress. As the machine become more efficient, it could posiibly integrated into wearable devices making it easier for us to detect early sign of stress and also possibly custom tailor a stress management solutions for user.

5.3 Market Potential

This project can be marketed for the public use by integrating the coding with smart wearable devices, highlighting its potential for real-time stress biomarker analysis. While acknowledging that further validation is needed to achieve medical-grade standards, this project will focus on building trust through transparency by collabarating with research institutions for validation studies, target health-conscious consumers and mental health professionals, and use social media and educational workshops to raise awareness. By gathering user feedback to refine the product, understanding regulatory requirements early, and showcasing success stories, this project can be position as a credible and innovative solution in the stress management market.

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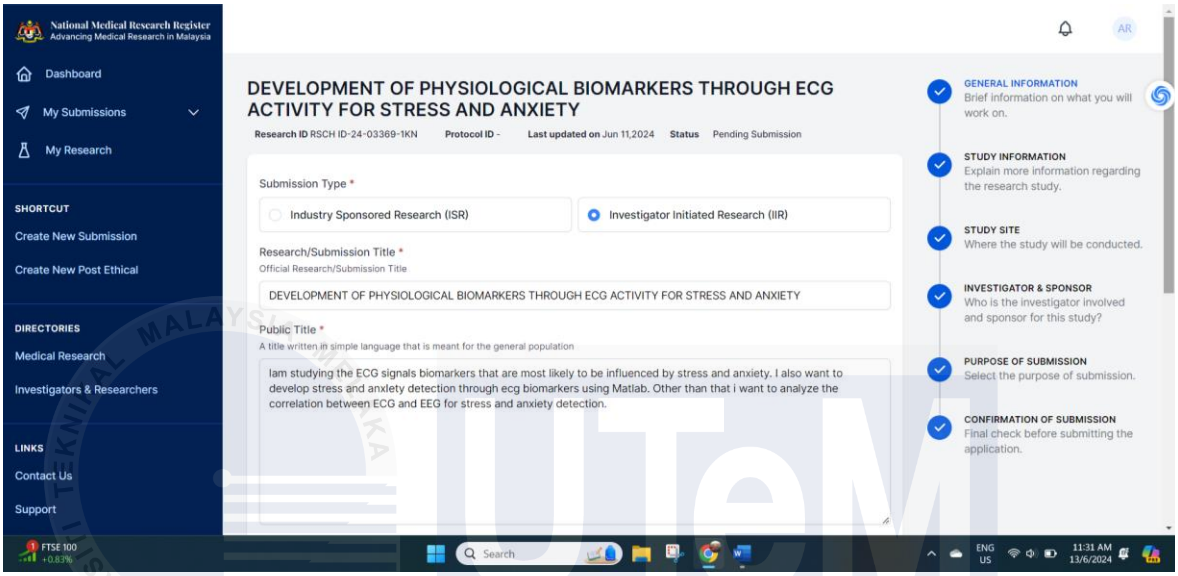
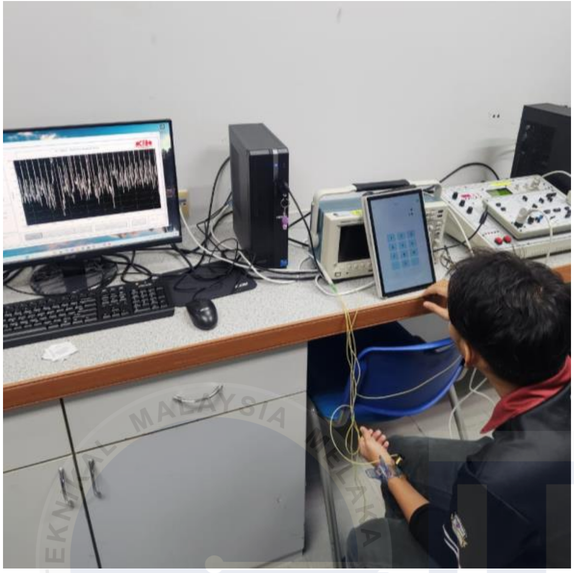
APPENDICES

Appendix A NMRR Registration



Appendix B Subjects During Data Acquisition



Appendix C   Stress And Non Stress Analysis

	Stress						
Dataset	Mean QT	Variance QT	Std Dev QT	Skewness QT	Kurtosis QT	Stress Episodes (QTc)	Stress Episodes (QTv)
Segment 1	0.20880	0.02259	0.15029	0.76816	2.09967	19	268
Segment 2	0.20344	0.02385	0.15443	0.82743	2.12666	17	266
Segment 3	0.19858	0.02202	0.14838	0.90003	2.32439	10	262
Segment 4	0.20208	0.00885	0.09410	-0.50034	2.83034	9	397
Segment 5	0.18902	0.00936	0.09677	-0.41233	2.41472	7	395
Segment 6	0.22121	0.00407	0.06381	0.92155	9.45688	8	239
Segment 7	0.20107	0.01653	0.12855	0.74519	2.58285	19	277
Segment 8	0.19775	0.01484	0.12180	0.74578	2.78406	22	402
Segment 9	0.19126	0.01166	0.10798	0.59266	2.80575	14	477
Segment 10	0.16886	0.00907	0.09525	0.37214	2.28949	10	393
Average	0.19821	0.01428	0.11613	0.49603	3.17148		

	Non Stress						
Dataset	Mean QT	Variance QT	Std Dev QT	Skewness QT	Kurtosis QT	Stress Episodes (QTc)	Stress Episodes (QTv)
Segment 11	0.18089	0.01103	0.10503	-0.36945	1.36437	0	374
Segment 12	0.21292	0.00992	0.09961	-0.36985	1.22176	0	404
Segment 13	0.17153	0.00729	0.08541	0.17620	1.38809	0	459
Segment 14	0.17296	0.00497	0.07049	-0.32152	1.57399	0	433
Segment 15	0.21484	0.00875	0.09352	-0.60554	1.45977	0	391
Segment 16	0.22198	0.00823	0.09073	-0.70912	1.57195	0	387
Segment 17	0.17641	0.01241	0.11140	0.48606	1.40810	0	327
Segment 18	0.31836	0.01999	0.14139	-1.19175	2.53736	0	241
Segment 19	0.28819	0.02256	0.15020	-0.72335	1.67753	0	241
Segment 20	0.31836	0.01999	0.14139	-1.19175	2.53736	0	241
Average	0.22764	0.01251	0.10892	-0.48201	1.67403		