ANALYSIS OF GLUTATHIONE SUPPLEMENTATION EFFECTS ON FEMALE METABOLIC SYNDROME CONDITION USING CLASSIFICATION TECHNIQUES

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FACULTY OF ENGINEERING
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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING SCIENCE

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ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Nur Rasyidah binti Hasan Basri

Matric No: KGA160047

Name of Degree: Master of Engineering Science

Title of Dissertation: Analysis of Glutathione Supplementation Effects on Female Metabolic Syndrome Condition Using Classification Techniques

Field of Study: Biomedical Engineering

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ANALYSIS OF GLUTATHIONE SUPPLEMENTATION EFFECTS ON FEMALE METABOLIC SYNDROME CONDITION USING CLASSIFICATION TECHNIQUES

ABSTRACT

Lesser known to the public that main antioxidants in our body, Glutathione are also said to influence the metabolic syndrome (MS) condition. Oral supplementation consisting of glutathione precursors and vitamin C introduced in the study was to improve glutathione (GSH) status of the consumer and the main objective of this study is to investigate GSH effects on selected MS condition. Several known studies had proven that oral supplementation improved GSH level of consumer. However, there is no definite proof on how it's affecting MS parameters. Also, there is less study carried out on the relationship between glutathione levels and metabolic syndrome especially among the Malaysian population. Prediction models will be constructed through classification technique to predict the MS parameter level based on GSH level and several other predictors. A randomized study was carried out on a total of 195 female volunteer subjects from Petaling Jaya, Malaysia and blood samples collected for analysis. Subjects were divided into 3 groups: Control group and 2 Intervention groups; Group 1 (consumed 1.6g of glutathione precursor's supplementation daily) and Group 2 (consumed 3.2g of glutathione precursor's supplementation daily). All data samples were used for model training purposes using three different classifiers; logistic regression, k-nearest neighbor, and decision tree). After 12 weeks, the supplementation were influencing the GSH level and some of the MS conditions, such as fasting glucose, triglycerides, LDL and total cholesterols showing that GSH alteration might closely related to the metabolic syndrome condition changes. Five predictors used in testing the significance of MS parameters is GSH, weight, body mass index (BMI) and waist hip ratio (WHR) and dosage groups.

From the results, multiple variables models were found to be significant on MS conditions compared to single variable. Model 1 to Model 3 with more than three combined predictors show significant results on glucose level and triglyceride cholesterols level at p value less than 0.05. Overall, prediction models through logistic regression classifiers performed the best in classifying the MS condition (glucose and lipid profiles) into normal and abnormal level at accuracy of more than 80%.

Keywords: Glutathione, Female, Metabolic syndrome, Classification

ANALISIS KESAN SUPLEMEN GLUTATHIONE PADA KEADAAN SINDROM METABOLIK PEREMPUAN MENGGUNAKAN TEKNIK KLASIFIKASI

ABSTRAK

Kurang diketahui oleh umum bahawa antioksidan utama dalam tubuh kita, Glutathione juga dikatakan mempengaruhi keadaan sindrom metabolik (MS). Suplemen oral yang terdiri daripada prekursor glutathione dan vitamin C yang diperkenalkan dalam kajian ini adalah untuk meningkatkan status glutathione (GSH) pengguna dan objektif utama kajian ini adalah untuk menyelidiki kesan GSH pada keadaan MS yang dipilih. Beberapa kajian yang diketahui membuktikan bahawa suplemen oral meningkatkan tahap pengguna GSH. Walau bagaimanapun, tidak ada bukti pasti mengenai bagaimana ia mempengaruhi parameter MS. Tidak banyak kajian dilakukan mengenai hubungan antara tahap glutathione dan sindrom metabolik terutama di kalangan penduduk Malaysia. Model ramalan akan dibina melalui teknik klasifikasi untuk meramalkan tahap parameter MS berdasarkan tahap GSH dan beberapa peramal lain. Satu kajian secara rawak telah dilakukan terhadap sejumlah 195 subjek sukarelawan wanita dari Petaling Jaya, Malaysia dan sampel darah dikumpulkan untuk dianalisis. Subjek dibahagikan kepada 3 kumpulan: Kumpulan kawalan dan 2 kumpulan Intervensi; Kumpulan 1 (mengambil 1.6g suplemen pendahulu glutathione setiap hari) dan Kumpulan 2 (menggunakan 3.2g suplemen pendahulu glutathione setiap hari). Semua sampel data digunakan untuk tujuan latihan model menggunakan tiga pengelasan yang berbeza; regresi logistik, jiran k-terdekat, dan pohon keputusan). Selepas 12 minggu, suplemen mempengaruhi tahap GSH dan beberapa keadaan MS, seperti glukosa puasa, trigliserida, LDL dan kolesterol total yang menunjukkan bahawa perubahan GSH mungkin berkait rapat dengan perubahan keadaan sindrom metabolik. Lima peramal yang digunakan dalam menguji kepentingan parameter MS adalah GSH, berat badan, indeks jisim badan (BMI) dan nisbah pinggul pinggang

(WHR) dan kumpulan dos. Dari hasilnya, model pelbagai pemboleh ubah didapati signifikan pada keadaan MS berbanding dengan pemboleh ubah tunggal. Model 1 hingga Model 3 dengan lebih daripada tiga ramalan gabungan menunjukkan hasil yang signifikan pada tahap glukosa dan tahap kolesterol trigliserida pada nilai p kurang dari 0.05. Secara keseluruhan, model ramalan melalui pengklasifikasi regresi logistik menunjukkan prestasi terbaik dalam mengklasifikasikan keadaan MS (glukosa dan profil lipid) ke tahap normal dan tidak normal pada ketepatan lebih dari 80%.

Kata Kunci: Glutathione, Perempuan, Sindrom Metabolik, Klasifikasi.

ACKNOWLEDGEMENTS

Deepest appreciation dedicated to my supervisors, Ir. Dr. Mas Sahidayana Mohktar, and Dr. Wan Safwani Wan Kamarul Zaman for the advice and guidance throughout the study. With their suggestion and encouragement, it contributed to the completion of the study and dissertation. I would also like to acknowledge with much appreciation the crucial role of University of Malaya Student Health Clinic staffs who gave the permission to conduct data collection there and participated in the study. Without clinic staff help, the study may have not be completed to this day.

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LIST OF SYMBOLS AND ABBREVIATIONS

MS	:	Metabolic Syndrome
GSH	:	Glutathione
BMI	:	Body mass index
WHR	:	Waist to hip ration
TC	:	Total cholesterols
TG	:	Triglyceride
HDL	:	High density lipoprotein
LDL	:	Low density lipoprotein
LR	:	Logistic regression
KNN	:	K-nearest neighbour

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Overview

Glutathione is a protein that naturally produce by body, consist of combination of amino acid; cysteine, glycine and glutamine. It is called 'master of antioxidant and detoxifier' as it important for body immune system and protecting cell from free radicals, thus making energy metabolism system regulate efficiently (Kern et al., 2011). Glutathione system in body is important as the antioxidant power help in inactive reactive oxygen species (ROS) that often occurs in individual with metabolic syndrome (MS) condition. This ROS have caused many chronic diseases such as diabetes mellitus, cardiovascular diseases and hypertension. A study by Hernandez et al., suggest that stimulation of antioxidant defense system which is glutathione system may become an important link between peroxidation and cholesterols metabolism. It shows that, stimulation of GSH activity in blood resulted in significant decrease in low density lipoprotein (LDL) and total cholesterols concentration but no significant changes on HDL and triglyceride concentrations (Hernández, Menéndez, & Wong, 1995). The existence of high glucose and cholesterols concentrations increase the reactive oxidative stress thus reducing the glutathione level in one's body (Avelar et al., 2015). There are limited studies on GSH relation with MS parameter, and results were hardly significant. On the other hand, previous studies were done mostly outside of Malaysia with data samples from Caucasian subjects. Hence, the study is focusing on to find the significance of GSH level on selected MS parameter which are glucose and lipid profiles (cholesterols) among Malaysian community.

Besides, it was previously reported that females have lower oxidative damage caused by ROS due to higher antioxidant activities. This is supported by the fact that females have higher glutathione level as compared to males (Borrás et al., 2003). Vina et al. (2011)

suggested that this happens due to ovarian hormones in females, which are oestrogens that help to regulate antioxidant activities in mitochondria (Vina et al., 2011). Thus, the study focused on female participants to observe the changes status of GSH level before and after GSH precursor's supplementations and its effects on improving the MS condition (glucose and lipid profiles) of its consumer.

Complex interaction of physiological data such as relationship between GSH and MS condition (glucose and lipid profiles) is the challenge that should be tackled in this study. Evaluating a regression models by estimating which variables that relevant for the MS condition are one of the way to provide clear insight of unknown or complex underlying physiology (Antoniewicz, Stephanopoulos, & Kelleher, 2006). Prediction models will be constructed through non-linear modelling technique with several predictor variables and one dependent variable called as response (Bernadó-Mansilla & Garrell-Guiu, 2003). Comparison of several classification techniques through different classifiers was also done to produce the best predicting models that can categorized GSH level into different classes (normal or abnormal) of MS condition.

1.2 Objective of Thesis

The objective of this thesis are:

i. To find the effects of glutathione (GSH) precursor's supplementation on female adult
ii. To classify and predict the MS parameters (glucose and lipid profiles) based on their GSH level and other predictors.

1.3 Hypothesis

MS is one of the factors that contributes to the formation of reactive oxidative stress in human body. As an antioxidant, glutathione plays a crucial role in reducing the effects of reactive oxidative stress. It was hypothesized that an oral supplementation of glutathione precursors' will improve the GSH level in the human body that has the ability to affect changes in glucose and lipid profiles.

1.4 Research significance

Due to limited previous work done on oral glutathione supplementation and results shown were hardly significant, this study is expected to produce significant changes of GSH concentration level after 12 weeks of GSH precursors' supplementation. Besides, the study focuses on only female participant as glutathione works more efficiently in female compared to male. Moreover, previous works were mostly conducted outside Malaysia, thus the data were unreliable to Malaysian community due to differences in diet, lifestyle and environment factors. Glutathione acts as antioxidant in reducing the oxidative stress produced by MS condition. Therefore, the relationship of glutathione status and some MS condition were investigated in this study. Then, model prediction will be constructed in classifying the MS condition based on the GSH level and several other health predictors. This method was used for ease of diagnosis or analysis on validating the effects of glutathione on MS condition, thus determining the status before and after the supplementation.

1.5 Scope of Work

The work scope of the thesis are as follows:

i. Data collection (consisting of glucose and cholesterols level from blood investigation) from healthy overweight female volunteers at University of Malaya

Student Clinic, Majlis Bandaran Petaling Jaya (MBPJ) Health Clinic and Tenaga Nasional Berhad (TNB) Dispensary, Bangsar.

ii. Determining the significance of GSH and some other predictors with glucose and cholesterols using single logistic regression and multiple logistic regression.

iii. Comparison of classification method through several classifiers to construct a prediction model of glucose and cholesterols level based on significant predictors.

1.6 Organisation of Thesis

Chapter 1 outlines general introduction, objectives and scope of work for the study.

Chapter 2 describes literature reviews on previous works in related fields of glutathione and MS condition. This chapter also describes relevant studies on non-linear modelling on various clinical application using classifications techniques such logistic regressions, k-nearest neighbor (KNN) and decision tree analysis.

Chapter 3 explains the detail of methodology used in the research. It covers data collection, blood investigation analysis, serum analysis and statistical analysis. Also included the implementation of logistic regression analysis to find the significant predictors to the MS condition. This chapter also comparing several non-linear classifiers in classifying best model to predict the condition of selected MS parameters.

Chapter 4 describes the descriptive results of blood investigation and GSH analysis. This chapter also observe the supplementation effects on volunteers MS condition after 4^{th}, 8^{th} and 12^{th} week. The optimum dosage that should be consume also discussed at the end of the chapter. The findings on significant predictors to MS condition also presented. Training prediction models constructed in classifying condition of MS based on several predictors. Model validation results displayed at final part of the analysis.

Chapter 5 discussed the significant changes after supplementation of glutathione on GSH level, and MS condition. The selection of predictors also discussed in this chapter. Several model constructed discussed to find best combination of predictors reliable in classifying glucose and lipid profiles into its normal and abnormal reading after supplementation.

Chapter 6 discussed overall conclusion, limitations and includes some suggestion for future works.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter discussed on previous research and studies carried out in relation to the study. In the earlier part, the importance of study on MS of Malaysia community were discussed and previous statistic on Malaysian data were shown. Then, the relation of glutathione and MS condition were explained for more understanding of the study objectives. Role of glutathione on human systems also presented in this chapter. From this Literature Review, the research gap was identified, and objective of the study was obtained. This chapter also discussed on the research methodology and analysis that will be conducted in this study.

2.2 Metabolic syndrome

Metabolic syndrome (MS) is a group of multiple risk factors including obesity, high triglycerides, low high density lipoprotein cholesterol (HDL-C), elevated blood pressure and high fasting blood sugar (Ghee, 2016). It is characterized by the presence of at least three of the five risk factors to determine one is suffering from the syndrome. Along with urbanization of the era, MS has secured a place worldwide as the number of people suffering from it rapidly increasing. This increase correlate to global epidemic of obesity and diabetes (Eckel, Grundy, & Zimmet, 2005). 23 percent of adults are at higher risk of cardiovascular disease, diabetes, stroke and diseases related to fatty build-ups in artery walls as the effect of MS. Therefore, MS is a serious health condition that should not be taken lightly.

2.2.1 MS in Malaysia

MS has affected 25 to 40 % of adult population in Malaysia between years of 2000 to 2015 based on databases of all literature with original data through search

databases(Ghee, 2016). Along with the modernization of era, people suffering from MS is rapidly increasing as a result of lifestyle, dietary and other environmental factors.

Previous studies discovers the prevalence rate of MS under the age of 40 are 25% and below however it rises to over 40% for population with the age of over 40 years old (Mohamud et al., 2012). Rampal *et al.* shows that MS also differ between genders which affected 30.1% among females and 24.1% among males(Rampal et al., 2012). Summary of the prevalence 'Ethnic Differences in the Prevalence of Metabolic Syndrome: Results from a Multi-Ethnic Population-Based Survey in Malaysia' were tabulated in Table 1. Previous study by Mohamud et al also found that women tend to suffer more from MS with 43.7% compared to men with 40.2% prevalent (Mohamud et al., 2012). In addition to that, a report from studies done in Kelantan, state of Malaysia, women indeed proven to have higher percentage in risk of MS (Zainuddin, Isa, Muda, & Mohamed, 2011).

Table 2.1: Prevalence of MS by age, gender and ethnicity among Malaysians ≥15 years(Rampal et al., 2012).

	Prevalence		
	Female	Male	Total
Age 15-40 years			
Malays	15.7	14.7	15.2
Chinese	13.9	13.4	13.7
Indians	23.3	21.8	22.5
Indigenous Sarawakians	26.3	22.1	24.2
Age >40 years			
Malays	51.5	38.5	45.0
Chinese	45.4	36.3	40.8
Indians	64.9	51.3	58.3
Indigenous Sarawakians	47.2	34.4	40.6

Malaysia is a multicultural country with population consists of three big ethnic group which are Malays, Chinese and Indians. Besides there are also presence of minor ethnic

groups in Sabah and Sarawak such as Iban, Melanau and others. Previous study also investigated on the MS rates between ethnicity. Shown that Indians have the highest rate of MS compare to other ethnic group (A. K. Tan, Dunn, & Yen, 2011). The study explain that Indians were hardly participate in physical activity and to consume less fruit and vegetables as the results of them being highest risk of MS compare to other ethnic.

2.2.2 MS symptoms and risk factors

The underlying causes of MS include overweight and obesity, physical inactivity, genetic factors and getting older. When a person has three or more symptoms stated in Table 2, the person suffers a MS and at risk for several metabolic diseases(Eckel et al., 2005)

Table 2.2: Metabolic syndrome symptoms (Eckel et al., 2005)

Metabolic syndrome symptoms	Men	Women
Waist Hip ratio	0.9	0.85
HDL cholesterol	>0.9 mmol/L	>1.0 mmol/L
Fasting glucose	≥5.6 mmol/L	
Triglyceride	≥ 1.7 mmol/L	
Blood Pressure	>140/90 mm Hg	
Obesity/Body mass index (BMI)	> 30	

Typically, when people suffer from MS, they will have apple-shaped bodies, like shown in Figure 1, larger waists and carry a lot of weight around their abdomens. Despite that, having a pear-shaped body, which carrying more of weight around hips and a narrower waist decrease the risk of diabetes, heart disease and other complications of MS (Clinic, 2016).

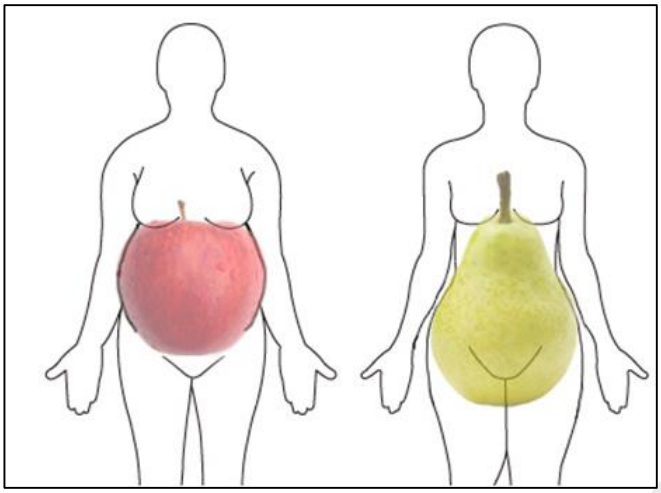

Figure 2.1: "Apple" and "Pear" body shape. (Mayo Clinic, 2018)

2.2.3 Obesity

Obesity is one of the risk factor in determining metabolic status of one's body health. Individual with body mass index (BMI) more than 30 are categorized into obese and likely to have high risk of suffering MS (Goodpaster et al., 2005).

In Malaysia, 27% of the adult population are overweight with BMI between 25.0 and 29.9; a further 5.8% have BMIs of >30. For both men and women age up to 50 years, there are progressive increment in weight therefore BMI with women achieving higher mean BMI. Between age of 20-29 years, 5-6kg in men and 6-7kg in women are likely to increase. Indian men and women showing the greatest increment compare to other ethnic. Obesity appears to be more prevalent in women than men and the rate in female Indians and Malays is particularly high (Ismail et al., 2002).

2.2.4 Blood Glucose level

Most widely used MS diagnostic criteria in international literature is represented by The Joint Interim Statement (JIS) (2009) and determines the level of blood glucose and waist circumference cut-off points. Although insulin resistance is not considered in the diagnosis of MS, it is a common condition, and several clinical manifestations of the

syndrome are explained from this dysfunction (Avelar et al., 2015). Previous study by Ford et al, insulin resistance is thought to be an underlying feature of the MS. Genetic abnormalities, fetal malnutrition, and visceral adiposity may play roles in the pathophysiology of insulin resistance and the MS. Although insulin resistance among patients with the individual components of the MS is common, significant proportions of these patients do not have insulin resistance (Ford, Giles, & Dietz, 2002).

2.2.5 Hypertension

One of risk factor that because MS are hypertension or elevated blood pressure. When overt hypertension is present without diabetes or chronic kidney disease, the goal for antihypertensive therapy is a blood pressure of <140/90 mm Hg. In the presence of diabetes or chronic kidney disease, the blood pressure goal is <130/80 mm Hg. Beyond these specific treatment goals, lifestyle changes deserve increased emphasis in people with the MS; the goals here are to reduce blood pressure as much as possible even in the absence of overt hypertension and to obtain other metabolic benefits of lifestyle change (Grundy et al., 2005).

2.2.6 Blood lipid level

The condition of abnormal levels of triglyceride and low HDL cholesterol are risks factors of MS. The normal cut-off level is >1.0 mmol/L and ≥1.7 mmol/L for HDL cholesterol and triglycerides respectively (Eckel et al., 2005).

2.2.7 Oxidative condition role in development of MS

Report from several previous studies shows that oxidative condition caused by the overproduction of reactive oxygen species (ROS) plays an important role in the development of MS. It also one of the main cause of related symptoms including obesity, hypertension, atherosclerosis, and type 2 diabetes mellitus (T2DM). The increment of

oxidative stress linked to decrease of antioxidant defense in our body. It can lead to body metabolic upsets and changes the cell signaling (Avelar et al., 2015).

The oxidative condition in MS may destruct the insulin signal pathway and lead to harmful action on the cell's endothelium. Thus, we can observe that this condition cause's insulin resistance appears to be a major cause of accelerated atherosclerosis and also may lead to development of T2DM. Individual with MS have elevated oxidative damage, as evidenced by decreased antioxidant protection that help to combat free radicals within the body that can damage the cells (Roberts & Sindhu, 2009).

Antioxidant or oxidant balance is well established as an important physiological regulator of arterial pressure and recently, its role in the pathogenesis of hypertension has been substantiated. Endothelial dysfunction is a cause of hypertension, mediated in part by oxidative stress, and antioxidants provide defense against vascular oxidative stress by neutralizing free radicals and protecting Nitrogen oxide (NO) from inactivation, thereby exerting beneficial effects on vascular function and structure (Roberts & Sindhu, 2009).

2.3 Glutathione

2.3.1 Role of Glutathione

Glutathione (GSH), a tripeptide antioxidant, is essential for cellular homeostasis and plays a vital role in diverse cellular. Glutathione is a cluster of amino acid containing one molecule of L-glutamic acid, L-cysteine, and glycine each. The molecule acts as an antioxidant where it is found in the food supply and in the human body. Supplementation of glutathione is thought to act as catalyst for the enzymes that synthesize glutathione within the cell to exerts antioxidant effects and thus maintain the efficacy of the entire glutathione system functions.

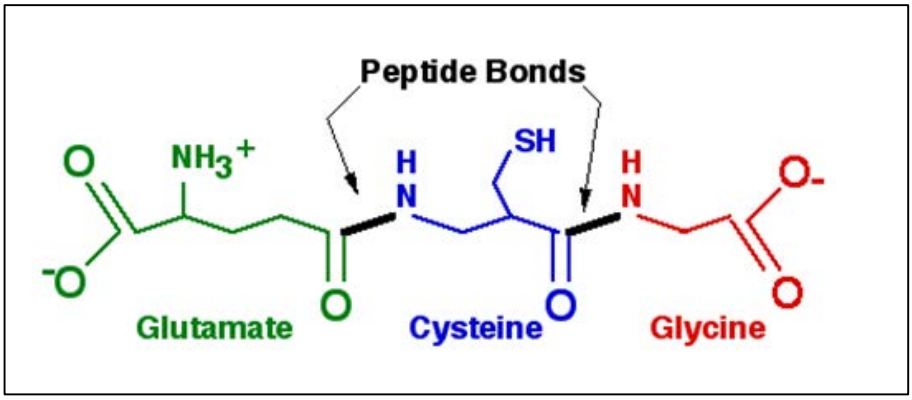

Figure 2.2: Glutathione (GSH) a tripeptide antioxidant. (MadSci Network, 2003)

The natural antioxidant system consists of numerous endogenous and exogenous components (acquired by diet) and antioxidant enzymes, which are able to inactivate ROS. The main enzymes participating in the oxidative stress reduction process are: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Avelar et al., 2015). Previous study shows that there are relations between the activities of antioxidant enzymes in accordance with number of MS individuals. From these results, we can argue that, in contrast to what has been described above, the mechanism used to prevent oxidative damage to cells would be to "strengthen" the body's frontline of antioxidant defense, which would result in increased activity of antioxidant enzymes in individuals with more severe metabolic changes (Yubero-Serrano et al., 2013).

2.3.2 Oral Glutathione precursors supplementation

Glutathione is an antioxidant that exist naturally in our body and also can be found in food supply such as vegetables and fruits. Supplementation of glutathione works as a catalyst for the enzymes that synthesizes glutathione to exert the antioxidant effect thus maintain the efficacy of glutathione system in our body. As antioxidant that act as a frontline defense to prevent oxidative damage to cells, increasing and maintaining the efficacy of glutathione system is important.

Figure 2.3: Food that rich in Glutathione (The Truth about Cancer, 2016)

2.3.3 Previous studies on Glutathione related to Oxidative stress

Previous study by Allen et al, who investigated on oral glutathione precursors supplementation on oxidative stress and GSH level, differences for observed changes in primary(baseline) or secondary endpoints (after 4 weeks) were without notable trend and did not reach statistical significance. Concentrations of reduced, oxidized, total (GSH + GSSG) and ratio measures of GSH status were unchanged by oral supplementation; $p > 0.05$ for each between-group comparison (Allen & Bradley, 2011). The results of this study determined that short-term, oral intake of GSH does not improve glutathione status, nor reduce markers of oxidative stress in healthy adults, and thus routine supplementation may not offer health benefits in the absence of disease or oxidative challenge.

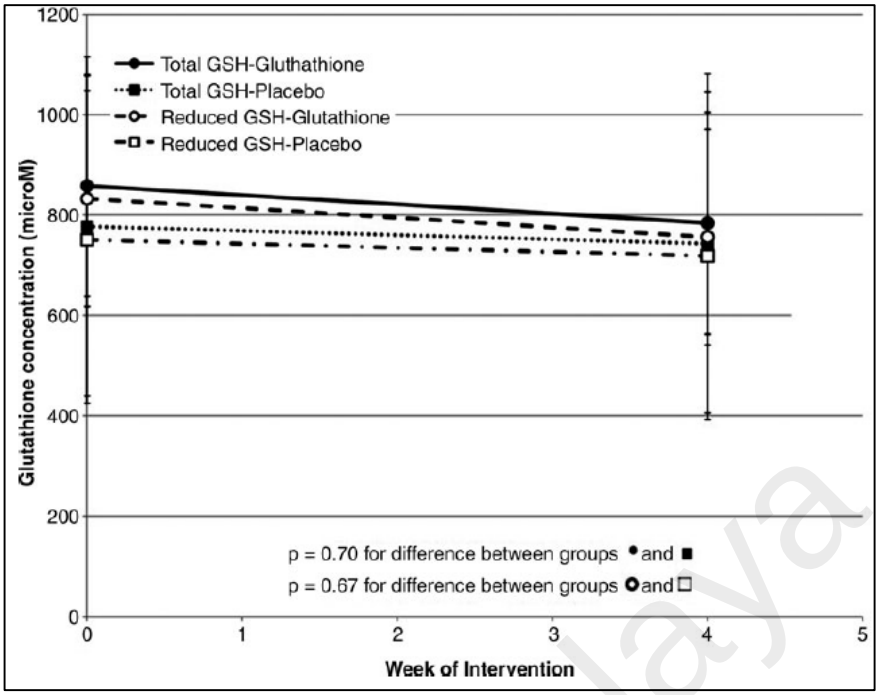

Figure 2.4: Effects of oral glutathione (GSH) on in vivo erythrocyte glutathione indices (Allen & Bradley, 2011)

Another study by John et al, the finding shows that for the first time, that daily consumption of GSH supplements of different dosage was effective at increasing body compartment stores of GSH. Study was conducted for a long-term supplementation period and GSH level were observe every first, third and sixth month after supplementation. GSH level in blood increases after supplementation month versus baseline for both doses in the study.

At 6 months, mean GSH levels increased 30–35 % in erythrocytes, plasma and lymphocytes and 260 % in buccal cells in the high-dose group ($P<0.05$). GSH levels increased 17 and 29 % in blood and erythrocytes, respectively, in the low-dose group ($P<0.05$). In most cases, the increases were dose and time dependent, and levels returned to baseline after a 1-month washout period. A reduction in oxidative stress in both GSH dose groups was indicated by decreases in the oxidized to reduced glutathione ratio in whole blood after 6 months (Richie et al., 2015).

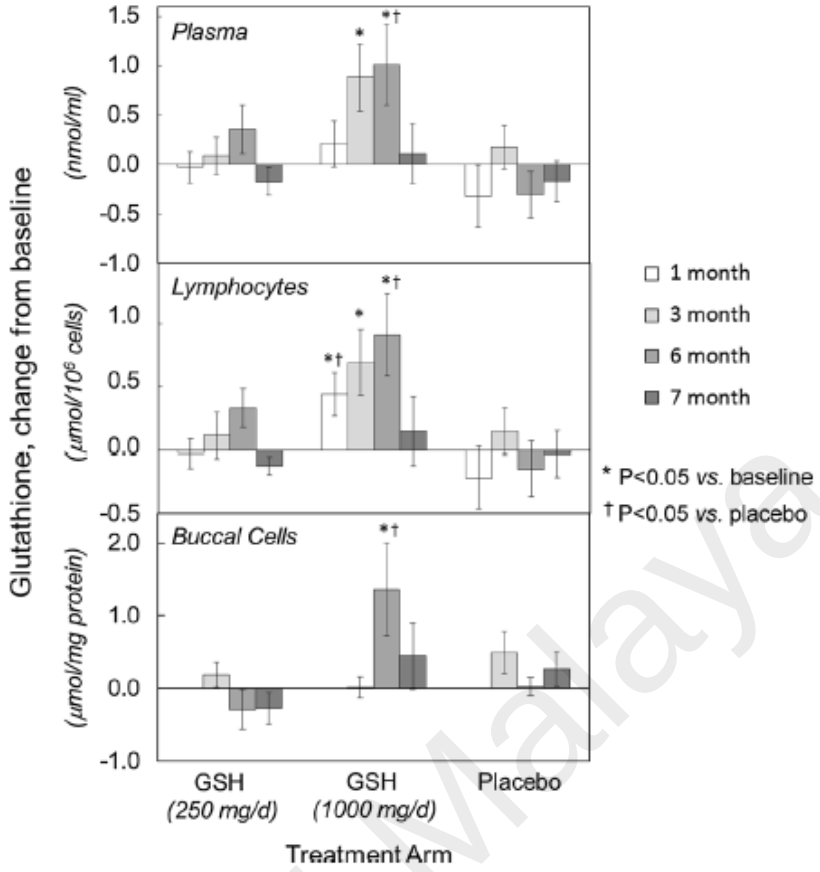

Figure 2.5: Effect of oral GSH precursors supplementationon total GSH in plasma, lymphocytes and buccal mucosa (Richie et al., 2015)

These previous works were conducted outside Malaysia, with difference environment and lifestyle. Therefore, the results were unreliable for Malaysian subjects. Besides, the uncertain results of oral glutathione supplementation are also one of the reasons that more study should be conducted on this topic. As the study aim to prove the relationship between glutathione and MS condition (glucose and cholesterols), using predictive data mining techniques enables the discovery of potential risk factors and can be used as clinical decision support system (Hassler, Menasalvas, García-García, Rodríguez-Mañas, & Holzinger, 2019). Predictive modelling were common techniques and widely used in medical data diagnosis. Big data and predictive analytics aid in making care management decisions leading to a stronger more motivational relationships between providers and patients (Menon, 2018). As in this case, between researcher and community. The study

using the existing collected data to train the model in classifying glucose and cholesterols level based on glutathione level and several other health predictors.

2.4 Engineering modelling technique

Complex interaction of physiological data such relationship between GSH and MS condition is the challenge that should be tackled in this study. Evaluating a regression models by estimating which variables that relevant for the MS condition are one of the way to provide clear insight of unknown or complex underlying physiology (Antoniewicz et al., 2006). Prediction models will be constructed through non-linear modelling technique with several predictor variables and one dependent variable called as response (Bernadó-Mansilla & Garrell-Guiu, 2003). This study focused on comparing several classification techniques through different classifiers to produce the best predicting models that can categorized GSH level into different classes (normal or abnormal) of MS condition investigated.

2.4.1 Non-linear modelling

Nonlinear regression is a form of regression analysis in which observational data are modelled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations. A mixed-effects model is a statistical model that incorporates both fixed effects and random effects. Fixed effects are population parameters assumed to be the same each time data is collected, and random effects are random variables associated with each sample (individual) from a population(Lindstrom & Bates, 1990).

An example of modelling the drug dosage, Pharmacokinetics. The purpose to understand intra-subject processes of drug absorption, distribution, and elimination governing achieved concentration and how these vary across subjects. Critical for

developing dosing strategies and guidelines. The setting of the study are 12 subjects with same oral dose (mg/kg) of Theophylline.

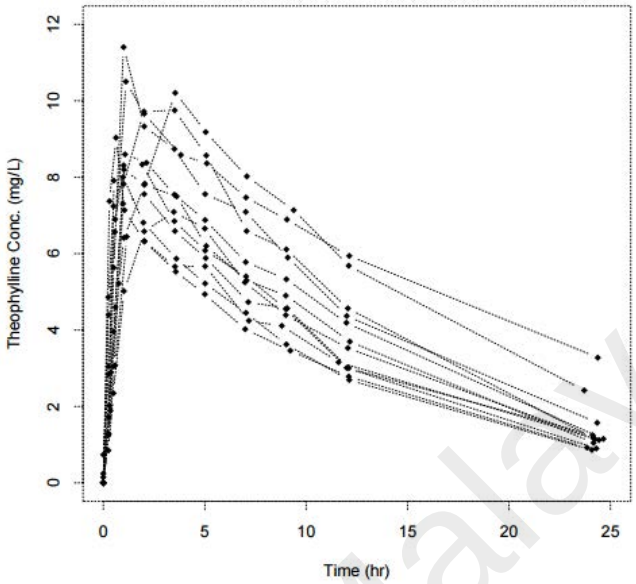

Figure 2.6: Nonlinear mixed effect model of the Pharmacokinetic.

Based on Figure 6, the model shows similarly-shaped concentration-time profiles across subjects but peak, rise, decay vary considerably. Attributable to inter-subject variation in underlying PK processes such as absorption, distribution and etc. This is an approximate representation of the body by simple compartment models (differential equations). It is a one-compartment model for theophylline following oral dose D at time t = 0 leads to description of concentration C (t) at time t ≥ 0. k_a, Cl, V summarize PK processes underlying observed concentration profiles for a given subject(Davidian, 2011).

$$C(t) = \frac{Dk_a}{V(k_a - Cl/V)}\left\{\exp(-k_a t) - \exp\left(-\frac{Cl}{V}t\right)\right\} \qquad \textbf{Equation 2.1}$$

K_a = fractional rate of absorption (1/time)

Cl = clearance rate (volume/time)

V = volume of distribution

To achieve study goal, more precisely stated to determine mean/median values of (k_a, Cl, V) and how they vary in the population of subjects. Elucidate whether some of this variation is associated with subject characteristics such as weight, age, renal function. Lastly to develop dosing strategies for subpopulations with certain characteristics for example dosage for the elderly(Davidian, 2011).

2.4.2 Type of classifiers

Most popular technique adapted in medical data analysis is logistic regression techniques. Most problem occur is there is no relationship between x (predictors) and y (response), therefore it has to be describe generally by probability distribution, P(x, y) (Bagley, White, & Golomb, 2001). Liao et al., reported that feature selection function in logistic regression analysis helps on reducing error in classifying the disease using genes as predictors (Liao & Chin, 2007).

Whereas, KNN classifier ranks the data's neighbours among the training data's, and use the class labels of k most similarity neighbours to predict the class of the input data (S. Tan, 2006). As reported by Sarkar et al., KNN technique was simpler and best to used compared to other technique in problem of diagnosis breast cancer (Sarkar & Leong, 2000).

Table 2.3: KNN classifiers attributes

Pre-set	K-nearest neighbour classifier		
	Fine	**Medium**	**Coarse**
No of neighbours	1	10	100
Distance metric	Euclidean	Euclidean	Euclidean
Distance weight	Equal	Equal	Equal
Standardize data	TRUE	TRUE	TRUE

Another simple and widely used technique in research is decision tree classification technique. It applies a straightforward idea to solve the classification problem. Previously

Gokgoz et al., achieved best performance on classifying electromyogram signals using decision tree algorithm with combination with random forest (Gokgoz & Subasi, 2015). The technique goes from observations of data which represented in the branches to conclusions represented in the leaves. Tree models where the target variable can take a discrete set of values are called classification trees; in these tree structures, leaves represent class labels and branches, or nodes represent conjunctions of features that lead to those class labels. The process involving steps of splitting the nodes, determining the terminal nodes and lastly assigning class to the terminal nodes. The class assigning is as simple as based on a weighted vote when assume certain classes is more likely than others (Pal & Mather, 2001).

Table 2.4: Decision tree classifier attributes

Pre-set	Decision Tree based classifier		
	Fine	**Medium**	**Coarse**
Max no. of split	100	20	4
Split criterion	Gini's diversity index	Gini's diversity index	Gini's diversity index

2.4.3 Model validation

To assess the quality of the model classification, two criteria needed to be evaluated which are discrimination and calibration. Discrimination is to shows how the data are separated well between classes while calibration determine accuracy of the probability prediction to the true probability. In small data cases, whole data will be split into pieces for training and testing. This process called n-fold cross validation. Common measures of the techniques are sensitivity, specificity and accuracy (Dreiseitl & Ohno-Machado, 2002).

2.5 Summary

Previous study shows adequate research were carried out on MS of Malaysian community. However, Malaysian were mostly unaware of the importance of glutathione on human body health. Glutathione are one of the important components to help in reducing the oxidative stress produced by MS diseases such as obesity and diabetes. As one of fattest community in South East Asia, studies related to MS topic may help in providing alternative method in improving the body health status of Malaysian community. Because of the limited researches and uncertain outcomes on this topic, this study proposes to conduct an assessment on a group of overweight female volunteers to consume Glutathione precursors' supplement, for at least 12 weeks or 3 months. The effect of different dosage of supplementation also will be investigated on these volunteers. As modelling techniques are commonly used in medical data diagnosis, constructing predictive model purpose to provide clear inside on the relationship between glutathione and MS condition (glucose and cholesterols). Previous report shows that random predictors associations resulting in different effects of parameter. In obesity and nutrition research, predictive modeling provided great value in many applications and is frequently used as data analysis technique (Ivanescu et al., 2016).

The study aims to prove the significance effects of oral GSH precursor's supplementation. The relationship between GSH level with glucose and lipid profile (Triglyceride, Total cholesterol, HDL and LDL) parameters were also investigated. Then, the classification of significant parameters based on several predictors including GSH will be done to construct best prediction model of the parameters. The data will be analyses with three different classifiers with outcome of selecting most suitable classifier that produce best prediction model based on its accuracy, sensitivity and specificity.

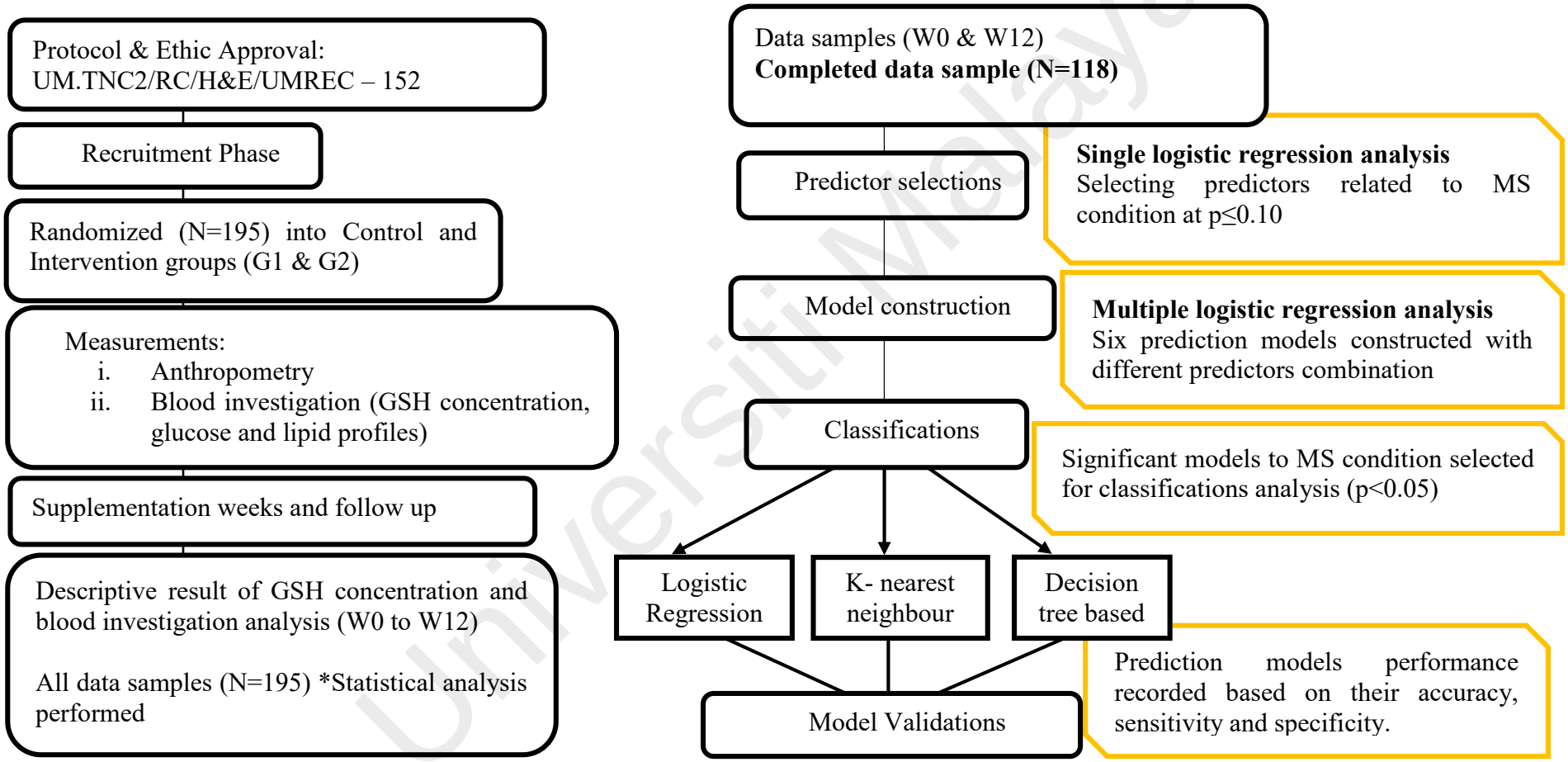

Figure 3.1: Flow chart summarizes the methodology of the study

3.1 Introduction

This chapter presented the procedure starting from first part of the study which consist of subjects' recruitment and data collection process. The second part presented on the analysis done; blood investigation on GSH level, fasting glucose and lipid profile before and after supplementation period. Final part was the construction of the model in classifying the MS condition into normal and abnormal classes.

3.2 Subjects recruitment

A total of 195 female adult volunteers have been recruited and participated in the present study. Subjects were recruited based on criteria; body mass index (BMI) > 24, aged from 18-65 years old with excluding criteria which are volunteers with chronic illness or pregnant/lactating mothers. The ethics and study protocol was approved by the University of Malaya Research Ethics Committee with reference number: UM.TNC2/RC/H&E/UMREC – 152.

3.3 Materials and Apparatus

Three TUD (Tactful, Unique and Dynamic, TUD Pte. Ltd. Malaysia) blood tubes, 5ml (with gel and clot activator), 6ml (plain with clot activator), 3ml (with K_2EDTA) and BD Vacutainer Safety-Lok blood collection needle set were used for blood collection. Glutathione Assay Kit (CS0260) from SIGMA-ALDRICH were obtained for GSH assay analysis. Apparatus used in the GSH assay analysis were 96 well plate, plate reader and multichannel pipette.

3.4 Data collection

The venue of data collection was divided into three; 1. University of Malaya Student Health Clinic (UMHC) and 2. Majlis Perbandaran Petaling Jaya (MBPJ) Health Clinic, Petaling Jaya and Tenaga Nasional Berhad (TNB) Dispensary, Bangsar. Subjects visited one of the clinics and blood samples were collected after receiving informed consent. All

subjects were briefly explained about the study and a written consent was obtained from each subject. Subjects were reminded to fast at least 8-12 hours prior the visits. Two types of measurements were performed: 1) anthropometric measurements; and 2) blood withdrawal for lipid profile test, hematology test (glucose) and GSH assay analysis.

3.4.1 Anthropometric measurements

The measurement includes measuring height, weight, body mass index (BMI) and waist hip circumference of the subjects. BMI was calculated by dividing subject's weight in kilogram (kg) by height in meters squared ($BMI = kg/m^2$). The procedure was performed before subjects went for blood withdrawal process.

3.4.2 Blood withdrawal for GSH analysis, Glucose and Lipid profile test

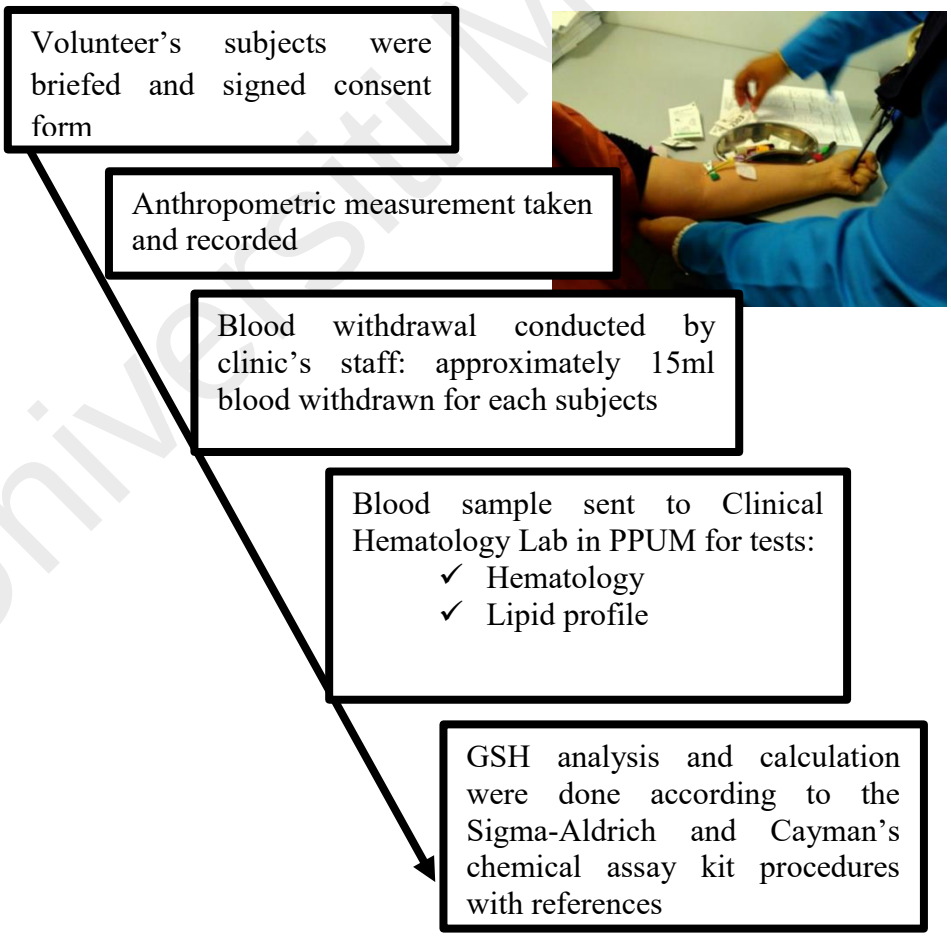

Figure 3.2: Flow chart of data collection protocol and blood investigation analysis

Approximate of 15ml blood was withdrawn from each subject for GSH analysis, hematology (glucose) and lipid profile test. 3ml blood was collected in purple and yellow TUD blood tube (with gel and clot activator) each was sent to Clinical Haematology Laboratory in University Malaya Medical Centre (UMMC) for tests. The blood withdrawal was carried out by professional medical staffs with the help of the research assistants.

Samples preparation for GSH analysis were conducted by research assistant in laboratory, another 5ml blood was collected in red plain blood collection tube with clot activator were centrifuged at 3000rpm for about 15 minutes to get the plasma serum. The plasma serum was pipetted into 1.5ml micro centrifuge tube PP (Tarsons Products Pte. Ltd., Kolkata, India) and stored into 2-8 °C freezer before further GSH assay analysis. GSH analysis and calculation were carried out according to the manufacturer's recommended protocol (Sigma-Aldrich (M) Pte Ltd, Kuala Lumpur, Malaysia).

3.5 Supplementation

Volunteer subjects were randomly categorized into control group and two intervention groups which undergoing the supplementation monthly. Control group consist of volunteers who normally proceed with their routine lifestyle and came for measurements for the baseline and 12 weeks from the first measurement. While, other two intervention group were schedule to come for baseline measurement and once a month assessment during the supplementation period. First group of supplementations (Group 1) were assigned to consume 5g of Immune Formulation 200®, Patent No: RE42645 E supplement (consist of 1.6 g glutathione precursors and vitamin C) while second group (Group 2) with two doses of 5g supplement daily (consist of 3.2 g glutathione precursors and vitamin C). The supplementation period also takes 12 weeks to complete for each subject. Every 4 weeks, the intervention group's subjects required to come for blood

withdrawal and anthropometric measurement. Figure 3.3 summarize the flow of the first part of this study; describing the recruitment, allocation and follow up process.

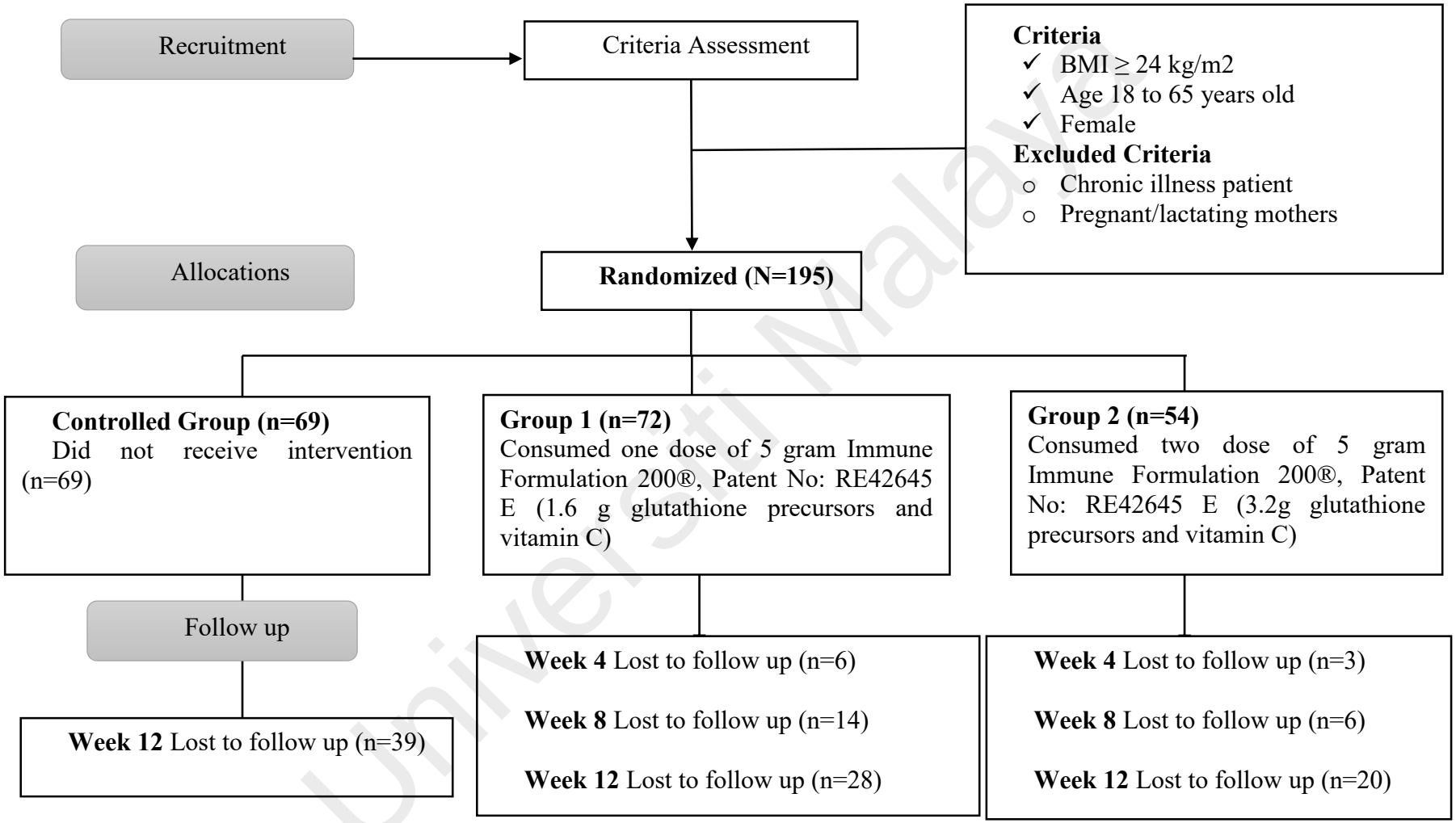

Figure 3.3: Flow chart of data collection procedure of the study

3.6 Statistical analysis

Statistical analysis performed using standard statistical analysis software (IBM SPPS Statistics 21, IBM Corp., United States) to evaluate the significant difference of the aforementioned blood component (glucose and lipid profiles) in control and intervention groups. A paired t-test analysis performed on blood tests and GSH analysis data to investigate the significant differences of the changes between baseline/week 0 and continuous weeks of supplementation (wee 4, week 8 and week 12). Differences with p value <0.05 were considered significant.

3.7 Classification analysis

Out of 195 subjects recruited, first 118 subject's data samples were used for model training purposes. The selected MS parameters investigated in this study were fasting glucose and lipid profiles and Table 3.1 summarize its range of levels with the conventional medical reference value. In further classification analysis, the numbers 0 and 1 will represent the normal and abnormal levels of the parameters respectively. By using classification application in MATLAB software Version R2018a (Math Works Inc., Massachusetts, United States), several classifiers used to construct predictions models for MS conditions based on several predictors, including GSH level. This study investigated the performance of three difference classifiers; which were logistic regression, k-nearest neighbor and decision tree-based classifiers. In the model construction, GSH, weight, BMI, WHR and supplementation groups will act as input predictors while MS parameters will become the outcome to be investigated

Table 3.1: Ranges of selected MS parameters

	Normal range	Abnormal range
Fasting Glucose	< 5.6 mmol/L	> 5.6 mmol/L
Triglyceride	< 1.7 mmol/L	> 1.7 mmol/L
HDL cholesterols	> 0.9 mmol/L	< 0.9 mmol/L
LDL cholesterols	< 2.59 mmol/L	>2.59 mmol/L
Total cholesterols	<= 5.2 mmol/L	>5.2 mmol/L

3.7.1 Input predictors and parameters selection

In this study, GSH level, weight, BMI and WHR acts as possible predictors to predict glucose and cholesterols level. Predictors were selected using multiple logistic regression method. Multiple predictors were combined and predictors with p value less than equal to 0.05 were considered for further analysis. The predictors were tested for correlation with glucose and cholesterols to find the significant value ($p < 0.05$).

3.7.2 Logistic regression analysis

It produces a logistic curve, which is limited to values between 0 and 1. The technique does not require a normally distributed or have equal variance predictors in each group. As data vectors x_i , the class probability of for the categories in data set was calculated using Equation 1 where $\alpha.x$ is a simple dot product for linear combination of vector components (Dreiseitl & Ohno-Machado, 2002). Removing excessive variables and leaving only most relevant as predictors can increase the accuracy of the models. In this study, one of three common approaches used which full model started with all predictors combined and remove one by one for the next models (backward selection). The significance of the models was tested to find best models whether more predictors produce better results or otherwise.

$$\boldsymbol{P(1|x,\alpha) = \frac{1}{1+e^{-(\alpha.x)}}} \qquad \textbf{Equation 3.1}$$

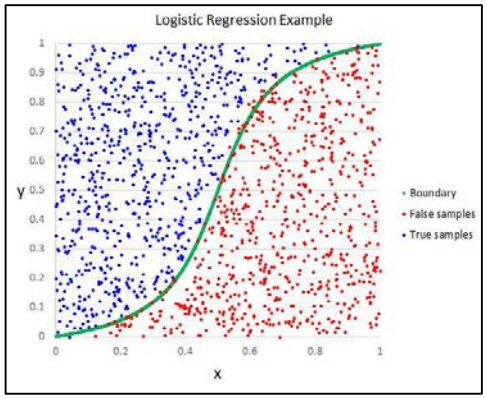

Figure 3.4: Logistic regression scatter plot example ("Logistic Regression (LR)," 2019)

3.7.3 K-nearest neighbour analysis (KNN)

Using KNN classification analysis, the method measured the Euclidean distance between test data and training data. The test data then was assigned to class label that most K closest training data have. The algorithm assumes data corresponds to points in N-dimensional space. The feature vectors are represented $(x_1^i, x_2^i, x_3^i, \dots, x_N^i)$ where x_k^i stand for kth attribute of the test datum x_i. The distance between x_i and x_j is calculated using Equation 2 (Sarkar & Leong, 2000). From the data, a model will be construct and new data will be classifies using the model. As an outcome, the present study aims to classify the level of GSH into several classes using the KNN classifier algorithm. The study uses no of neighbour k=1 as attribute for the classifier.

$$\mathbf{d(x_i, x_j)} = \sqrt{\sum_{\mathbf{k=1}}^{\mathbf{N}} (\mathbf{x_k^i} - \mathbf{x_k^j})^2} \qquad \textbf{Equation 3.2}$$

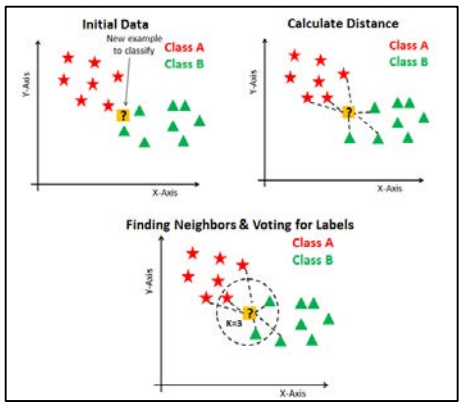

Figure 3.5: K-nearest neighbour classifications (Navlani, 2018)

3.7.4 Decision tree analysis

The decision tree inducing algorithm must provide a method for specifying the test condition for different attribute types as well as an objective measure for evaluating the goodness of each test condition. For decision tree-based analysis, no of split of 100 used as classifier attribute in this study with split criterion of Gini's diversity index.

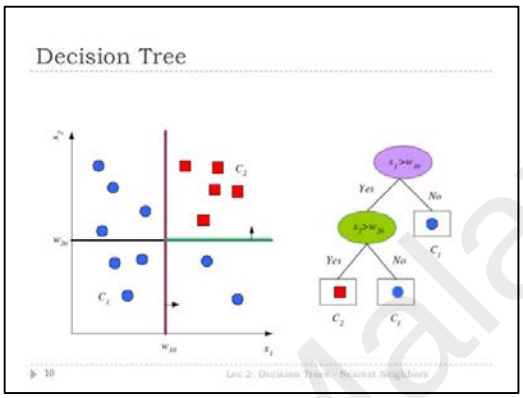

Figure 3.6: Decision tree classification (Gavin, 2016).

3.7.5 N-fold Cross-validation

In testing the accuracy of the classifier, the predictive models evaluated by partitioning the original sample into a training set to train the model, and a test set to evaluate it. In n-fold cross-validation, the original sample is randomly partitioned into n equal size subsample (Dreiseitl & Ohno-Machado, 2002). In this data training, 10 folds of cross validation was used to cross validate the training set. 108 subjects' data samples used for training while 10 data from the training population were set aside for validating or testing the models. The models will be evaluated based on their performance on accuracy, sensitivity and specificity.

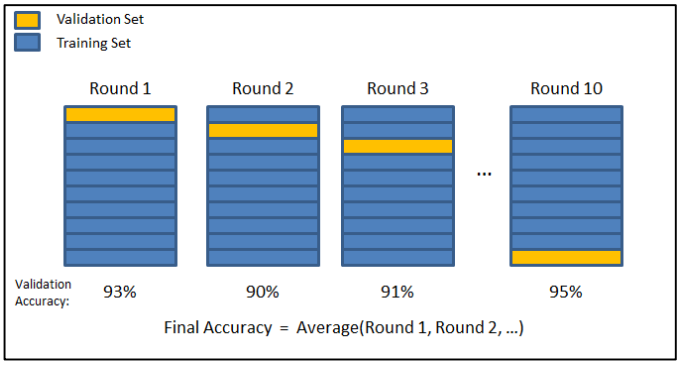

Figure 3.7: Cross validation steps example (Drakos, 2019)

CHAPTER 4: RESULTS

4.1 Introduction

This chapter presented findings of the study which divided into two parts. Part A represent the results of supplementation of GSH on MS conditions while Part B showed models construction on glucose and lipid profiles classification.

4.2 Part A: Descriptive results on GSH concentration and blood investigation

First part analysis of the study was observation on GSH concentration and blood investigation (glucose and lipid profile) during baseline and continuous supplementation weeks. Total of 195 subject's results were observed and reported.

Table 4.1: Demographic data of randomized subjects grouping. Data are mean±SD.

	Control (n=69)	Group 1(n=72)	Group 2(n=54)
Age	36.88±9.76	37.56±10.49	38.30±9.13
Height	1.57±0.06	1.57±0.09	1.56±0.06
Weight	71.24±12.19	74.32±15.50	75.04±13.25
BMI	28.82±5.16	30.40±7.74	31.01±5.59
WH ratio	0.86±0.05	0.86±0.08	0.86±0.05

Demographic data of randomized subjects grouping shown in Table 4.1, displayed subjects aged average within 30 years old and weighing more than 70 kg. Crucial criteria for the study was the BMI, as all subjects recruited recorded more than 25 kg/m^2 marking the possibilities of having MS condition.

4.2.1 GSH concentration level

Table 4.2: Total GSH concentration of Control and Intervention groups. Data are mean±SD.

GSH (µM)	Week 0	Week 4	Week 8	Week 12
Control	13.32±5.59			12.00±4.31
Group 1	13.10±8.36	**19.22±24.01***	**16.45±9.64***	**16.55±10.62***
Group 2	13.10±5.57	12.93±4.85	12.53±6.61	14.54±5.78

Notes:*Significant at $p<0.05$

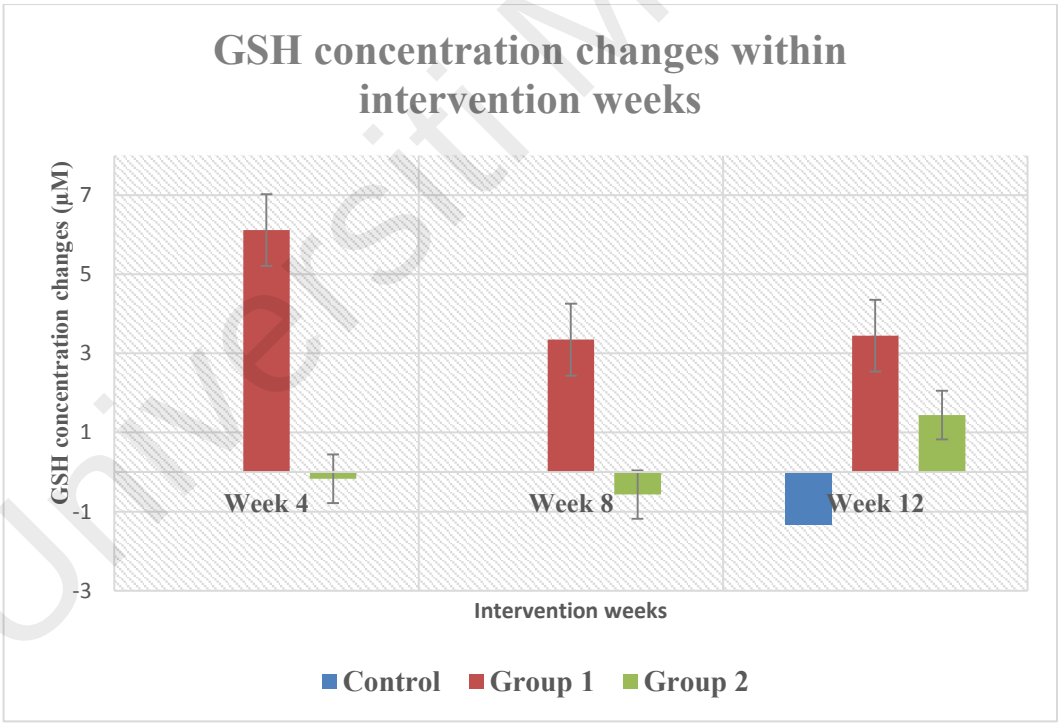

Figure 4.1: Total GSH concentration changes between baseline/week 0 and intervention weeks.

Table 4.2 recorded the results of total GSH concentration of each groups during the baseline and intervention weeks. At baseline/Week 0, all groups recorded almost similar total GSH level which at average of 13 μM. Fluctuations in GSH concentration occurred in subjects of the intervention groups. Figure 4.1 visualized the changes occurred during the intervention weeks. Group 1 experienced a significant increment of GSH concentration level with 6.12, 3.35 and 3.45 μM increment at Week 0, Week 8 and Week 12 respectively. While Group 2 suffers slight fluctuations whereby the GSH level rose higher than its baseline value (Week 0) at Week 12 with 1.44 μM increment. From the graph, Group 1 experience immediate spiked changes of GSH concentration in week 4 and continue to stabilize in week 8 and week 12. However, Group 2 only showed slightly changes in week 4 and week 8, but started to increase at week 12. Theses fluctuations occurrence might suggest that Group 2 subjects needed more time to adapt with the dosage compared to Group 1 subjects in affecting the GSH level after supplementation.

4.2.2 Fasting glucose level

Table 4.3: Fasting glucose of Control and Intervention groups. Data are mean±SD.

Fasting Glucose (mmol/L)	**Week 0**	**Week 4**	**Week 8**	**Week 12**
Control	4.96±1.22			4.76±0.49
Group 1	5.22±2.20	5.16±1.92	5.18±1.75	5.25±1.74
Group 2	5.47±1.58	**5.35±1.40***	**5.31±1.60***	5.56±1.95

Notes:*Significant at $p<0.05$

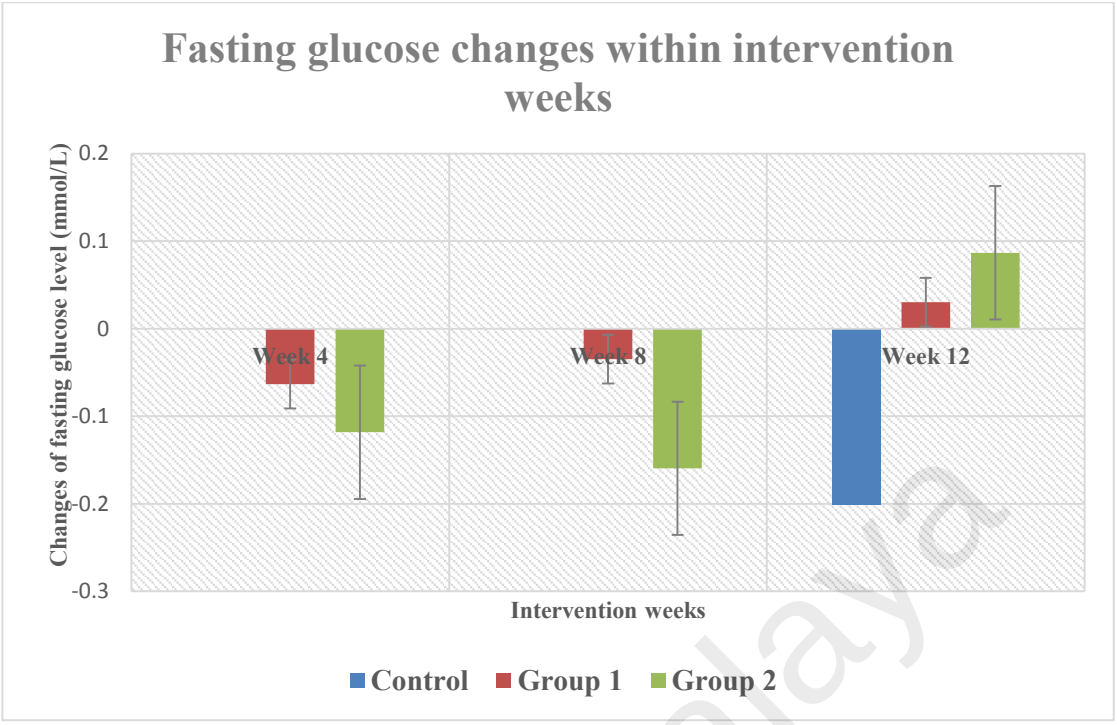

Figure 4.2: Fasting glucose changes between baseline/week 0 and intervention weeks.

Table 4.3 recorded the fasting glucose level of each groups during the study period. All three groups started at borderline of fasting glucose normal reading which is less than 5.6 mmol/L. The changes can be observed in Figure 4.2 where intervention groups showed improvement during Week 4 and Week 8 compared to baseline (Week 0). However, for Group 1, it was a slight decrement thus not significant. Group 2 recorded a significant decrement at Week 4 and Week 8 with 0.12 and 0.16 mmol/L respectively. Yet, fluctuations occurred at Week 12, where intervention groups suffer slight increment in glucose level while Control group recorded a decrement of 0.2mmol/L compare to its baseline value.

4.2.3 Lipid profile (cholesterols) levels

Table 4.4: Lipid profile (Cholesterols) levels of Control and Intervention groups. Data are mean±SD.

	Triglyceride (normal: <1.7mmol/L)				**Total Cholesterols (normal: ≤5.2 mmol/L)**				**HDL (normal: >0.9 mmol/L)**				**LDL (normal: <2.59 mmol/L)**			
	Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12	Week 0	Week 4	Week 8	Week 12
Control	1.14± 0.55			1.12± 0.44	5.16± 0.78			5.19± 0.63	1.43± 0.36			1.30± 0.27	3.24± 0.69			3.38± 0.51
Group 1	1.33± 0.81	1.19± 0.60	**1.13± 0.51***	**1.20± 0.59***	5.26± 0.93	5.36± 0.88	5.15± 0.88	5.16± 0.88	1.35± 0.32	1.38± 0.28	1.34± 0.25	1.32± 0.24	3.30± 0.79	**3.44± 0.77***	3.31± 0.80	3.30± 0.79
Group 2	1.21± 0.56	1.15± 0.49	1.28± 0.75	1.19± 0.75	5.33± 0.82	5.20± 0.83	**5.19± 0.79***	5.38± 0.86	1.39± 0.29	1.35± 0.29	**1.35± 0.30***	**1.38± 0.31***	3.39± 0.69	3.32± 0.71	**3.25± 0.66***	3.47± 0.70

Notes:*Significant at $p<0.05$

Table 4.4 recorded the lipid profile reading of control and intervention group's subjects. Lipid profile consist of four cholesterols level reading which are triglyceride, total cholesterols, HDL and LDL levels. These cholesterols levels were investigated on each group for 12 week period within the study. For triglyceride cholesterols, intervention groups showed slightly higher value during baseline compared to Control group. Similar cases displayed in total cholesterols reading, where intervention groups started slightly higher than normal range (>5.2mmol/L) while Control group was at the borderline of normal reading. HDL and LDL cholesterols reading showed consistent value throughout the study with slight fluctuations during intervention weeks for all groups.

Figure 4.3 displayed the changes of triglyceride cholesterols level during the intervention weeks compared to baseline week. Group 1 showed consistent decrement throughout the study. Significant decrement of 0.2 and 0.14 mmol/L recorded at Week 8 and Week 12. Fluctuations occurred in Group 2 where its reading reduced at Week 4, abrupt increment at Week 8 and continued to reduce slightly at Week 12. Nonetheless, the fluctuations occurred range between 0.02 to 0.06 mmol/L, thus not affecting much on the normal ranges of triglyceride levels.

Changes of total cholesterols level were displayed in Figure 4.4. As reported, intervention groups recorded slightly higher total cholesterols level to normal ranges compared with Control subjects at baseline week. As Control subjects showed insignificant changes after 12^{th} weeks, yet intervention groups recorded some improvement with the total cholesterols reading. During the 4^{th} week, Group 1 showed slight increment with 0.1mmol/L but counter the reading after Week 8 and Week 12 with decrement of 0.1mmol/L, thus helping these subjects achieving the normal total

cholesterols level. For Group 2 where, the total cholesterols level significantly decrease right after Week 4 and Week 8 with 0.14 and 0.15 mmol/L, however suffered slight increment at Week 12 with 0.05mmol/L. Group 2 subjects did achieved the normal ranges of total cholesterols during Week 4 and Week 8 but this was not achievable in Week 12.

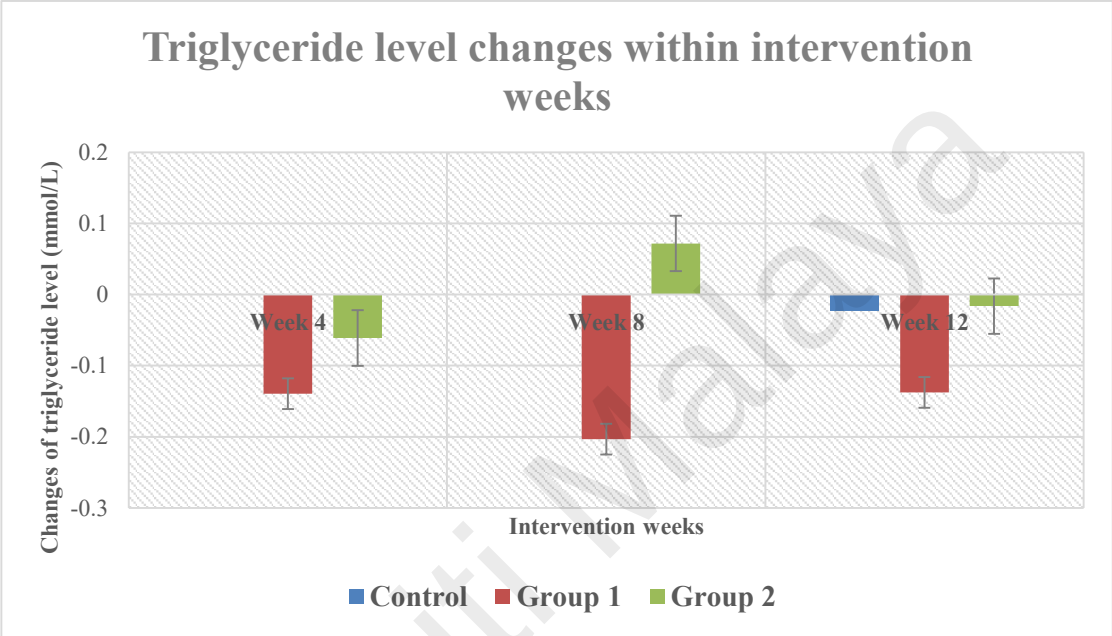

Figure 4.3: Triglyceride level changes between baseline/week 0 and intervention weeks.

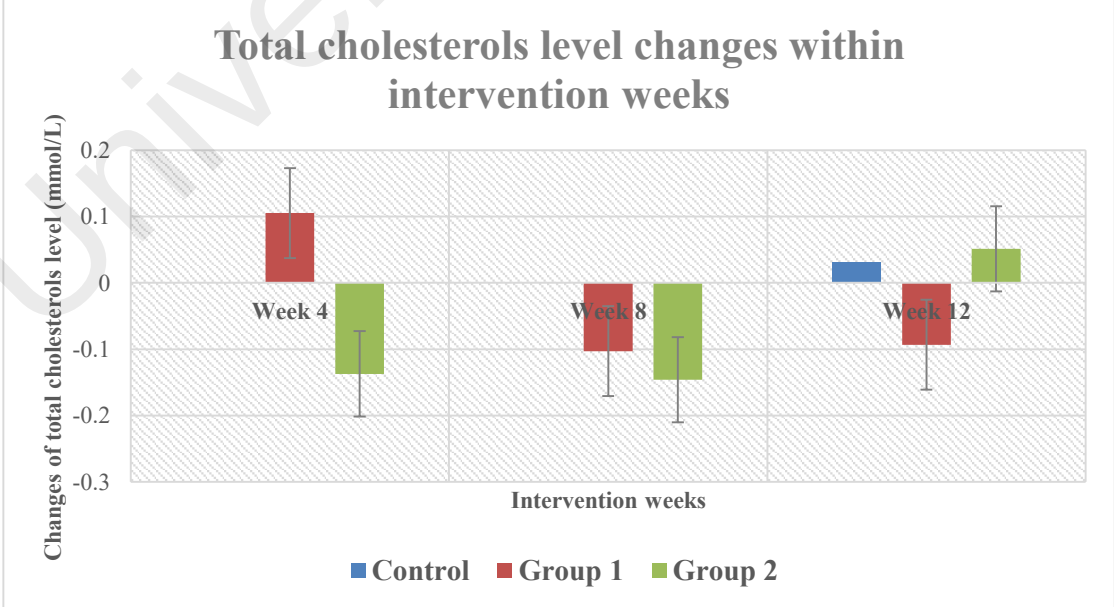

Figure 4.4: Total cholesterols level changes between baseline/week 0 and intervention weeks.

Changes of high density lipoprotein (HDL) cholesterols were shown in Figure 4.5. Slight fluctuations observed in intervention groups results thus not affecting the ranges of normal and abnormal of HDL cholesterols level throughout the study. HDL cholesterols was expected to be increase as it describes as 'good' cholesterols in blood, which reducing the risk of heart disease. Despite of that, only Group 1 was observed to have an increase in level with slightly at Week 4 with 0.03mmol/L. At week 8 and Week 12, both intervention groups suffered slight decrement. HDL level of Control group also decreased at final week of the assessment. Throughout the study, all groups maintained in the normal ranges of HDL cholesterols level despite the decrement.

Figure 4.6 displayed the low density lipoprotein (LDL) cholesterols level of the intervention week compared to its baseline week. LDL cholesterols is described as a 'bad' cholesterols thus should not be present in blood more than 2.59 mmol/L. From Table 4.4, the reading of LDL cholesterols for all groups during baseline (Week 0) were exceeding normal ranges with average of 3.3 mmol/L. During Week 4, Group 1 recorded increment of 0.14 mmol/L but improve at Week 8 with slight increment of 0.01 mmol/L and maintained the same value as baseline at Week 12. Whereas Group 2 showed gradual significant decrement at Week 4 and Week 8, but suffered slight increment at Week 12. Despite the changes, LDL cholesterols level of volunteers in Group 2 were not able to achieve normal ranges even after the 12^{th} weeks of supplementation period.

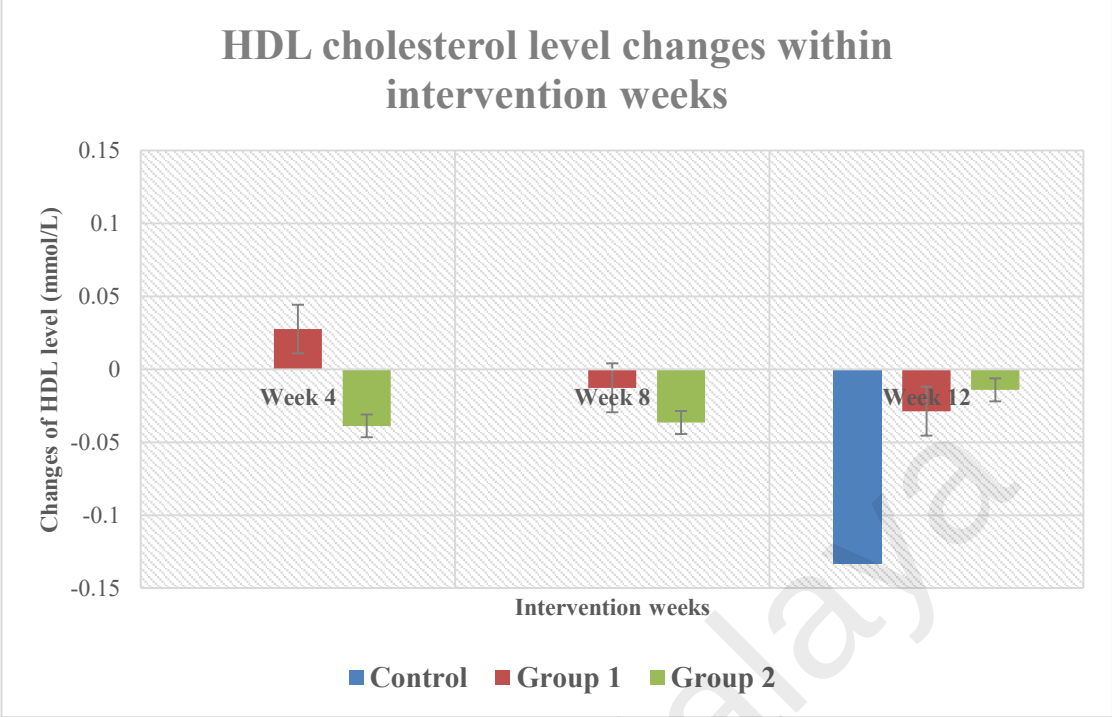

Figure 4.5: HDL cholesterols level changes between baseline/week 0 and intervention weeks.

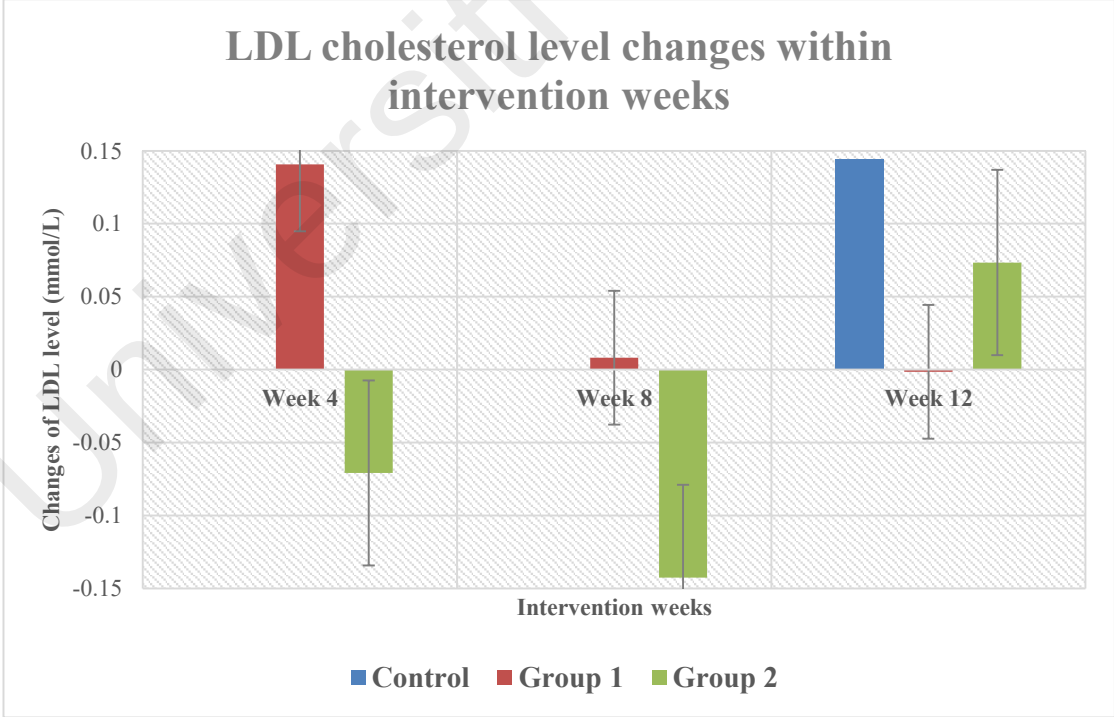

Figure 4.6: LDL cholesterols level changes between baseline/week 0 and intervention weeks.

From Part A results, the supplementation were influencing the GSH level and some of the MS conditions, such as fasting glucose, triglycerides, LDL and total cholesterols. These results shows that GSH alteration might closely related to the MS condition changes. The study continue to find whether this GSH can become a reliable predictor in predicting the level of glucose and lipid profiles with other selected health predictors such as BMI, WHR, and weight.

4.3 Part B: Classification of MS condition based on GSH, weight, BMI, WHR and supplementation groups.

Second part of the study was construction of prediction models to classify the MS condition into normal and abnormal classes based on several predictors selected including GSH. Figure 4.8 summarized the flow of procedure starting from collecting completed data samples (Week 0 and Week 12) until model construction, classification and validation. From Part A, most subjects dropped out or lost to follow up after several weeks of assessment. Therefore, only 118 completed subject's data sample will be analyzed in Part B.

Table 4.5: Anthropometric data samples on MS conditions. Data are mean±SD.

Variables (Unit)	Glucose (mmol/L)		TG (mmol/L)		HDL (mmol/L)		LDL (mmol/L)		TC (mmol/L)	
	Normal (n=98)	Abnormal (n=20)	Normal (n=46)	Abnormal (n=72)	Normal (n=114)	Abnormal (n=4)	Normal (n=22)	Abnormal (n=96)	Normal (n=55)	Abnormal (n=63)
Weight (kg)	71.96 ± 12.54	80.66± 17.34	73.24± 14.64	66.71± 10.52	73.40± 13.78	75.29± 18.89	73.49± 16.02	71.93± 18.21	72.61± 13.40	72.50± 13.42
BMI (kg/m^2)	29.25 ± 5.93	33.94± 7.15	30.09± 6.80	27.09± 4.30	30.06± 6.38	29.00± 7.74	29.75± 6.63	29.13± 7.48	29.32± 5.36	29.29± 5.38
WHR	0.86 ± 0.07	0.89± 0.06	0.86± 0.07	0.83± 0.05	0.86± 0.07	0.88± 0.01	0.85± 0.04	0.83± 0.14	0.85± 0.04	0.85± 0.04
GSH (µM)	13.10 ± 6.18	14.69± 8.87	12.80± 5.97	12.59± 6.21	13.08± 6.11	32.01± 16.36	12.48± 8.89	12.39± 8.66	13.13± 7.46	13.10± 7.49

Table 4.5 recorded the whole population data samples during baseline assessment on their MS condition classes. MS condition were divided into two classes which are normal and abnormal classes. Classes were divided according to conventional medical reference value summarized in Table 3.1. In further analysis, the numbers '0' and '1' will represent the normal and abnormal levels of the conditions, respectively.

4.3.1 Significant predictive variable selection using single logistic regression (SLR) analysis

Table 4.6: p-Values from single logistic regression (SLR) analysis.

Predictors	MS condition				
	Glucose	**TG**	**TC**	**HDL**	**LDL**
GSH	0.17	0.12	0.08	**0.05***	0.32
Weight	**0.02***	**0.007***	0.08	0.54	0.49
BMI	**0.006***	**0.05***	0.21	0.23	0.88
WHR	**0.005***	0.19	0.12	0.4	0.21
Groups	**0.007***	**0.04***	0.75	0.92	0.73

Notes:*Significant at $p<0.05$

Selection of predictors were done through single logistic regression analysis where each predictor was correlated with each MS condition and reliable p-values were observed to proceed with the investigation. Predictors with p-value of below and equal to 0.05 were proven significant to the MS condition. From table 4.6, all predictors demonstrate significant value to at least one of the MS conditions. Therefore, this may suggest that this predictors might contribute some effects on the reading of MS condition throughout the study. Predictors selected to proceed with model constructions were GSH, weight, BMI, WHR and supplementation groups.

4.3.2 Predictive model constructions using multiple logistic regression (MLR) analysis

Six models were constructed on baseline data using multiple logistic regression. Removing excessive variables or predictors and leaving most relevant can increase the accuracy of the models. In this study, one of the three common approach implemented starting with constructing full model with all predictors and individually removed predictors for the next model (backward selection). For baseline model construction, the grouping predictor was excluded to investigate whole population MS conditions before they were allocated into their respective groups.

Table 4.7: p-Values from multiple logistic regression (MLR) analysis on baseline assessment.

WEEK 0/ Baseline		**Glucose**	**Triglyceride**	**Total Cholesterols**	**HDL**	**LDL**
Model 1	GSH WHR Weight BMI	**0.0001***	**0.05***	0.21	**0.002***	0.55
Model 2	GSH WHR BMI	**0.00009***	**0.004***	**0.027***	**0.004***	0.83
Model 3	GSH BMI	**0.008***	**0.014***	0.29	**0.003***	0.9
Model 4	GSH Weight	**0.04***	**0.03***	0.18	**0.002***	0.92
Model 5	GSH WHR	**0.01***	0.082	0.16	**0.003***	0.45
Model 6	GSH	0.26	0.2	0.67	**0.0004***	0.96

Notes:*Significant at $p<0.05$

From table 4.7, Model 1 to Model 6 shows significant correlation to at least one of the MS conditions. Full model or Model 1 with GSH, WHR, weight, and BMI as its predictor

showed significant correlation to Fasting glucose, triglyceride and HDL cholesterols. Whereas Model 2 also showed correlation with those three MS condition with additional of total cholesterols. Model 3 and 4 displayed similar results as full model despite differences in its predictor's combination. While, Model 5 and Model 6 both showed least correlation with only two and one MS condition respectively.

Table 4.8: p-Values from multiple logistic regression (MLR) analysis on final assessment.

WEEK 12		Glucose	Triglyceride	Total Cholesterols	HDL	LDL
Model 1	GSH WHR Weight BMI Groups	**0.02***	**0.004***	0.83	**0.001***	0.17
Model 2	GSH WHR BMI Groups	**0.04***	**0.0002***	0.56	0.07	0.18
Model 3	GSH BMI Groups	**0.013***	**0.05***	0.53	0.09	0.52
Model 4	GSH Weight Groups	**0.05***	0.15	0.89	**0.012***	0.48
Model 5	GSH WHR Groups	0.48	**0.021***	0.85	**0.047***	0.25
Model 6	GSH Groups	0.85	0.64	0.9	**0.05***	0.88

Notes:*Significant at $p<0.05$

For final assessment (Week 12) model construction, supplementation grouping predictors was added to investigate whether different dosage of supplementation affecting the classification of MS conditions. Therefore, additional groups' predictor were combined with existing predictors in Model 1 to Model 6. From table 4.8, with additional predictors, Model 1 still correlated with fasting glucose, triglyceride and HDL cholesterols. Whereas

Model 2 and 3 both displayed correlation with fasting glucose and triglyceride. In the other hand, Model 4 exhibit correlation with fasting glucose and HDL. Least correlation occurred to Model 5 and 6 where both models only match to one MS condition which was HDL.

From both baseline and final assessment model construction, all prediction models showed significant correlation at p value <0.05 to these three MS condition; fasting glucose, triglyceride and HDL cholesterols. This shows that these predictors were influencing those three MS condition, while total cholesterols and LDL might be less affected by combination of GSH, WHR, BMI, weight, and supplement dosage throughout the study. Therefore, these prediction models might be reliable to classify the normal and abnormal levels of glucose, triglyceride and HDL cholesterols. To conclude the findings on which models will accurately classify the level of MS condition, the performance of their accuracy, sensitivity and specificity will be investigated.

4.3.3 Predictive models' classification and validations

Models construction were proceeded with classification of MS condition with different classifier techniques; logistic regression, k-nearest neighbor and decision tree based classifier. Data samples in these models were used for training and testing purposes. The models then will be evaluated based on their performance on accuracy, sensitivity and specificity. Accuracy in this study define as model's ability in predicting subjects MS conditions level into normal and abnormal class correctly, whereas sensitivity display its ability to predict normal level and specificity to predict abnormal level correctly. In this part of analysis, although from the models construction, HDL were influenced by the combination of predictors, but because of the abnormal data distribution of HDL, where most subjects recorded normal HDL level throughout the study, HDL cholesterols

parameter were dropped out to avoid biased data samples that will be affecting the accuracy and specificity of the prediction models.

4.3.3.1 Baseline: Week 0

The comparison of baseline model classification accuracy were tabulated in Table 4.9 and Table 4.10. Table 4.9 displayed the performance of baseline models classification on fasting glucose levels for three different classifiers. Model 1 to Model 3 exhibit best performance with logistic regression classifier as the accuracy and sensitivity for those three models exceeded 80% while specificity of the models was slightly lower at range of 50%. From the results, logistic regression classifier was able to produce prediction models with the ability to predict subjects with normal glucose correctly at a higher accuracy yet still lacking to predict the subjects with abnormal glucose. On the other hand, Model 4 to Model 6 shows best performance with different classifier which was the complex tree classifier. Although the accuracy of the models were lower compared to logistic regression classifier, this complex tree classifier managed to displayed better results on sensitivity and specificity where logistic regression failed to do so. Though, the specificity percentage was so low that may suggest that Model 4 to Model 6 might not suitable in predicting glucose level of the subjects.

Table 4.10 showed the performance of baseline prediction models through three different classifiers on triglyceride cholesterols level. Model 1 to Model 6 except Model 5 all showed their best performance through logistic regression classifier. Though the accuracy of the models were not the best, they manage to achieve 50% to 60 % accuracy with sensitivity and specificity range between 50 to 70%. Whereas Model 5 shows best performance with k-nearest neighbor classifier to produce better sensitivity percentage nearly 50%. However, from the performance, Model 1 to Model 4 were consider good

enough in predicting triglyceride levels compare to other two models. Its ability to correctly classify normal and abnormal triglyceride level were better though less accurate compared to glucose.

Table 4.9: Performance of baseline prediction models for fasting glucose level. Data are in percentage,%.

Glucose models	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
Logistic regression	83.9	87	55	83.9	86	57	83.1	84	50	83.1	83	0	81.4	83	0	83.1	83	0
KNN	78	85	29	78	87	35	72.9	82	13	66.1	80	5	74.6	84	22	66.9	82	12
Complex tree	80.5	86	40	78.8	86	35	74.6	83	19	69.5	83	17	78	85	31	74.6	83	14

TG; triglyceride cholesterols, KNN; k-nearest neighbour. Highlighted are the best classifier performance of each models constructed

Table 4.10: Performance of baseline prediction models for triglyceride cholesterols level. Data are in percentage,%.

TG models	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
Logistic regression	69.5	68	70	65.3	59	67	64.4	59	66	63.6	57	65	58.5	38	61	57.6	25	60
KNN	55.9	44	65	51.7	40	62	61	50	68	44.9	31	55	55.1	41	62	42.4	28	53
Complex tree	58.5	47	67	60.2	49	68	61.9	51	68	52.5	38	61	47.5	30	56	42.4	21	53

TG; triglyceride cholesterols, KNN; k-nearest neighbour. Highlighted are the best classifier performance of each models constructed.

4.3.3.2 Final assessment: Week 12

The comparison of final assessment model classification accuracy were tabulated in Table 4.11 and Table 4.12. Performance of final assessment prediction model for fasting glucose were displayed in Table 4-11. After 12th week of supplementation period, another predictor added into the models which was the grouping that indicate dosage difference in supplement consume by the subjects. The performance of the models also changed after supplementation. Model 1 and Model 6 showed best performance with k-nearest neighbour classifier by producing 80% accuracy and sensitivity yet lower specificity especially Model 6 with 24%. Instead, logistic regression were best performed in Model 3 and Model 4 with mostly 85% accuracy and sensitivity and higher specificity which Model 3 achieved 100% while Model 4, 50%. Model 2 and Model 5 both performed their best with complex tree classifier to produce 80% of accuracy and sensitivity with slightly lower specificity ranges between 35 to 50%. For fasting glucose level prediction model at final assessment, Model 3 showed best performance compare to other models with highest accuracy and good specificity and sensitivity percentages.

Table 4.12 tabulated the performance of final assessment prediction models for triglyceride levels cholesterols level. For this MS condition, after the supplementation, Model 1 to Model 3 showed best performance through logistic regression classifier. Although the accuracy (ranges from

60 to 70%) may not consider the best, it is good enough in classifying normal and abnormal triglyceride level after the supplementation period. The sensitivity and specificity of the results were sufficient to prove the model ability to classify correctly according to its classes. Whereas Model 4 to Model 6 exhibited its best performance through complex tree classifier with Model 5 and Model 6 achieving more than 80% accuracy. Yet for the triglyceride prediction models, Model 1 to Model 3 were better compared to others though lower in accuracy. This was due to better results in sensitivity and specificity, where the models are able to correctly classify subjects with normal triglyceride level and subjects with abnormal triglyceride level after the supplementation.

Table 4.11: Performance of final assessment prediction models for fasting glucose level. Data are in percentage,%.

Glucose models	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
Logistic regression	83.1	85	40	83.1	85	40	85.6	85	100	83.9	84	50	83.9	84	0	83.9	84	0
KNN	84.7	89	54	76.3	87	29	78.8	86	31	79.7	87	35	76.3	87	29	76.3	85	24
Complex tree	76.3	86	26	83.1	87	45	78	84	18	81.4	85	33	81.4	87	38	76.3	83	9

KNN; k-nearest neighbour. Highlighted are the best classifier performance of each models constructed.

Table 4.12: Performance of final assessment prediction models for triglyceride cholesterols level. Data are in percentage,%.

TG models	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity
Logistic regression	65.3	60	68	69.5	66	72	66.1	63	68	54.2	43	58	87.3	0	87	87.3	0	87
KNN	63.6	57	69	56.8	49	63	52.5	44	59	48.3	38	55	80.5	17	88	87.3	25	88
Complex tree	60.2	53	64	59.3	53	63	55.9	48	61	56.8	49	62	84.7	29	88	83.9	50	90

KNN; k-nearest neighbour. Highlighted are the best classifier performance of each models constructed.

4.4 Summary

In summary, after 12th week of supplementation period, there were significant improvement in GSH level of intervention groups subjects, thus correlated with the changes of MS condition investigated in the study which were, fasting glucose, triglyceride, total cholesterols, HDL and LDL cholesterols. Alteration of GSH level influenced the changes of MS condition, especially in fasting glucose, triglyceride and HDL. Therefore, GSH was investigated as predictors in prediction models with other health predictors such as BMI, weight and WHR in predicting the normal and abnormal MS conditions. Predictors were selected and combined to construct model prediction on classifying MS condition during baseline (Week 0) and final assessment (Week 12). Models then trained and tested through three different classifiers, then validated with accuracy ranges between 60 to 80%.

CHAPTER 5: DISCUSSIONS

5.1 Introduction

MS had affected 25% to 40 % of adult population in Malaysia between the year 2000-2015 based on databases of all literature with original data through search databases (Ghee, 2016). Reactive oxidative stress (ROS), occurring in subjects, caused these MS condition to emerge. Previous study had proven that patients with MS and Type 2 diabetes mellitus increase their oxidative stress production (Spanidis et al., 2016). Previous report by Michelet et al. (1995) proved that plasma GSH showed no significant difference in gender influence (Michelet et al., 1995). However, other studies stated that GSH activities in females were significantly higher as compared to males. However, the level of GSH concentration was unlikely to show significant results between gender; thus, further research should be done on this aspect(Habif et al., 2001; Liu, Harrell, Shenvi, Hagen, & Liu, 2005) . Borras et al. (2003) proved that mitochondrial GSH levels were higher in female as compared to males, and thus reduce the peroxidation occurrence in females. These conditions were helped by the presence of oestrogen hormones that prevent the damage to the cells(Borrás et al., 2003). Therefore, GSH affect women more as compared to males.

Proven that glutathione works more efficiently in female, the study purposed to investigate the effects of oral glutathione supplementation in improving GSH level and its relationship to the MS condition of its consumer. From the data collected, predictive model constructed in classifying the MS parameter (glucose and cholesterols level) based on glutathione level and several other health predictors. This method aid in diagnosing the MS condition of subjects based on their glutathione level.

5.2 Part A: Descriptive results on GSH concentration and blood investigation

5.2.1 GSH concentration level

After 12 weeks of supplementation, there were changes observed in intervention groups subjects regarding their GSH concentration levels. Group1 subjects who consumed one dose of supplement daily (consist of 1.6 gram glutathione precursor's) showed immediate improvement of GSH concentration level after week 4. Different occurrence happened for Group 2 subjects who consumed two dose of 5g glutathione supplement daily, where they suffer slight decrease during first 4 and 8 weeks, but manage to counter during week 12 by rose higher compared to its baseline value. This occurrence suggested that subjects in Group 2 required certain time to be adapted with two dosage of the supplementation compared to Group 1 subjects. According to previous study, a notable trend and significant changes on effect of oral GSH precursor's supplementation are hardly observed in a short-term of study period. Study by Allen et al, proved that after 4 weeks of oral supplementation two dose of 0.5g glutathione capsule daily, the GSH status in blood were unchanged thus did not reach statistical significance (Allen & Bradley, 2011). Therefore, this study approached a longer supplementation period and investigated the differential in dosage effects on GSH concentration of blood plasma. Another study from John et al, increased in plasma GSH level occurred after three and 6 month of supplementation of 1g glutathione, with concentration ranges between 0.2 to 8 nmol/ml(Richie et al., 2015). However, in this study, the GSH concentration level in blood plasma of these subjects were higher ranging from 2 to 15 μM during baseline and increasing to almost 20 μM during the intervention weeks. The results also show significant increment with $p<0.05$ especially in Group 1, noted that the supplementation affects the changed in GSH level. The dosage of glutathione precursors was higher in this study thus resulting in higher GSH level after supplementation. Another difference found in this study was supplementation used was in powdered form while

both previous study mentioned were providing supplementation in capsule form. As widely known powders are undoubtedly easier to be absorbed by the body compared to pills or capsule. Bioavailability of powdered supplementation was greater compared to capsules or tablets. These have been proven by published report on the bioavailability of supplementation forms (Hartman-Craven, Christofides, O'Connor, & Zlotkin, 2009). The differences in GSH concentration value between the study and previous reports may due to this occurrence. However, further analysis should be done to provide more conclusive reasoning.

5.2.2 Fasting glucose level

Along with the improvement of the GSH concentration level, the fasting glucose level in blood also showed some significant changes. While control group maintained no changes to its glucose reading, intervention groups recorded an improvement in reducing the readings after some time of supplementation. As one of MS condition, fluctuation of glucose level in blood often related to the increment of reactive oxidative stress in our body. Glutathione system acts as frontline to utilize its antioxidant power in controlling the level of this reactive oxidative stress. Therefore, during the study period, supplementation improved the GSH system efficacy by increasing the concentration of GSH level in blood plasma thus indirectly helping in reducing the level of blood glucose of its consumers. Previous report also published that GSH improvement did contribute to significantly improve glucose disposal rate and insulin sensitivity (Nguyen, Hsu, Jahoor, & Sekhar, 2013).

5.2.3 Lipid profile (cholesterols) levels

Another MS conditions investigated related to GSH improvement were blood cholesterols level. Control group recorded no significant changes in all cholesterols component after 12th week assessment. Whereas, intervention groups showed some

significant changes in each cholesterols component investigated. There are limited published reports on GSH relation to cholesterols level. From previous study, higher cholesterols level also associates with the increment of oxidative stress. High cholesterol levels trigger mitochondrial oxidative stress on cartilage cells, causing them to die, and ultimately leading to the development of osteoarthritis (Farnaghi et al., 2016). Function of GSH system in the body are crucial to counterattack the damage done by reactive oxidative stress. A study by Maciel et al, suggest that the absence of glutathione enzymatic activity was associated with hypertriglyceridemia and low HDL-cholesterol levels in humans(Maciel et al., 2009). Supported by these reports, results shown in this study suggest that glutathione precursor's supplementation played a major role in improving the cholesterols level of the subjects. Especially on triglyceride and total cholesterols components. Both intervention groups managed to reduce the total cholesterols level to normal range (<5.2mmol/L) within the study period. Yet, the changes were not significant.

5.3 Part B: Classification of MS condition based on GSH, weight, BMI, WHR and supplementation groups

5.3.1 Significant predictive variable selection using single logistic regression (SLR) analysis

In selecting predictors, single logistic regression analysis performed to selected variables. From previous discussion, GSH concentration suggested closely relate to changes in MS condition, thus considered one of the possible predictors in constructing prediction model. On the other hand, having higher BMI always leads to a risk of suffering from health problems. Main criterion of recruiting subjects in this study is having BMI more than 24, which represents overweight to obese volunteers. Weight and waist to hip ratio also often used in assessing health status of human body. Having heavier weight and unbalance WHR contributed to increasing risk of having MS condition.

5.3.2 Predictive model constructions using multiple logistic regression (MLR) analysis

A differential in GSH dosage might affects the MS conditions after the 12th week assessment. However, from the results the significant value of models changes after addition of groups predictors. From previous studies, they investigated the effects of supplementation of 1000mg or 1g or lower (Allen & Bradley, 2011; Kern et al., 2011; Richie et al., 2015). In this study, the dosage investigated were 5g and 10g of supplementation. Therefore, the results may differ and contribute to changes of significant value in constructing prediction models.

According to the results, for baseline models, Model 2 with combination of GSH, WHR and BMI correlated with most MS conditions as compared to others. BMI proves its relation to glucose and triglyceride also total cholesterols of the volunteers. The results recorded p value of less than 0.05. A study on Malaysian population by Termizy et al. (2009) found that obese patients (BMI>30) raised fasting blood glucose by 17% and reduced HDL to 40%. They also stated that these patients had higher risk of suffering from MS (Termizy & Mafauzy, 2009). At 12 weeks of assessment, Model 2 exhibited different results in correlation to the MS condition. After certain supplementation period, due to fluctuations of the MS conditions and addition of groups predictors might affect the significant value of the model performance.

Weight and WHR are also important in determining one's health besides BMI. However, its power is reduced when it stands alone as a predictor. A company of BMI needs to show better results of the relation with MS condition. From the results, weight was affecting more on glucose level while WHR had some relation with glucose as well as triglyceride. As discriminators for MS condition, WHR appeared to be less useful as compared to BMI. A similar finding was shown in a study by Cheong et al. (2015) where

BMI and waist circumference were reported to have better discrimination ability on predicting components of MS (Cheong et al., 2015).

5.3.3 Predictive models' classification and validations

Models constructed were put through different classifiers to assess its ability in classifying the MS condition of normal and abnormal levels. The data were train and tested to produce predictive models based on different combination of predictors. From the model construction results, most models displayed significant relationship with fasting glucose, triglyceride and HDL cholesterols ($p<0.05$). However, from the demographic data of study, the models prediction on HDL cholesterols might be inaccurate due to biased data distribution. Results showed having more predictors in a model helped in increasing the correlation with the conditions. As full model or Model 1 and Model 2, and Model 3 showed significant relationship with glucose and triglyceride cholesterols during both baseline and final assessment. For the classifying power, Model 1 to Model 3 worked best with logistic regression classifier in exhibiting accuracy of more than 80% with high sensitivity and specificity in classifying glucose level at baseline level. However, at final assessment, the results differs for each models. Yet, logistic classifier still showed good performance but not the best. From the results, it can be concluded that Model 1 to Model 3 are reliable enough in predicting the subjects' glucose level based on different combination of the predictors. While the models were reduced by its predictors, the performance also diminished. This may be the reasons why Model 4 to Model 6 were displaying lower performance compare to Model 1 to Model 3 for both baseline and final assessment. Similar to glucose, triglyceride cholesterols also best predicted with Model 1 to Model 3 through logistic regression classifier. Though the accuracy was lower for triglyceride classification, but it still considered reliable in predicting the level of triglyceride before and after the supplementation based on combination of GSH, WHR, BMI, weight and differential in dosage predictors. As most

popular technique adapted in medical data analysis, logistic regression once again proved its ability in classifying glucose and triglyceride cholesterols level of glutathione precursor's supplementation consumers.

5.4 Summary

The GSH level of female subjects significantly ($p<0.05$) increase after 12 weeks of oral glutathione precursor's supplementation. The study period was sufficient in observing the changes of GSH status in intervention groups' subjects. However, in observing the supplementation effects in human subjects, a longer term of research required to produce more conclusive and accurate results. Due to time and budget limitation, the study managed to observe until the 3rd month (12 weeks) of supplementation to conclude with the results. Despite that, the desired results shown, thus supported the hypothesis of the study. Data collected from the blood investigation and GSH analysis were used in constructing predictive model to classifying MS condition into normal and abnormal level. Using this method, useful predictors were determined in diagnosing MS condition. Models were tested through several classifiers to provide most accurate and precise outcome.

CHAPTER 6: CONCLUSIONS AND FUTURE WORKS

6.1 Summary

Overall, oral supplementation was observed to improve the GSH concentration status of the consumers, especially with one dose of 1.6g glutathione precursor's supplementation. Subjects consuming two doses of 3.2g glutathione precursor's supplementation did improved a bit later at week 12. This suggested that subjects in this group required some time to be adapted with the environment changes of the system with additional dosage of the supplementation. In relation to MS condition, intervention groups' subjects displayed some significant changes after some time. Improvement recorded for fasting glucose, triglyceride and total cholesterols level after certain weeks of supplementation. Noted that subjects in these group manage to reduce total cholesterols into normal range after 12^{th} week from their baseline reading. From the observation, one dosage of 5g Immune Formulation 200®, Patent No: RE42645 E supplement (consist of 1.6g glutathione precursors and vitamin C) showed to be an optimum dosage that can improved the status of glutathione concentration thus indirectly helping to enhance positive metabolic condition.

Selected predictors investigated appeared to be significant at least to one MS condition in the study. BMI, weight, WHR, GSH and dosage groups were recorded to have correlation with glucose, triglyceride cholesterol at p value <0.05 at baseline and final assessment. Therefore, from the study we conclude that these predictors are affecting MS condition in female volunteers. Having higher level of these predictors may increase the risk of having abnormal levels in either glucose, triglyceride cholesterols. Models construction showed that, all predictors played a role in determining the normal and abnormal levels of MS conditions. Reduction in one predictor might be a cutback for the classifying process performance. Models with more predictors exhibit more reliable performance compare to model with one or two predictors. Among the classifiers, logistic

regression classifiers displayed the best performance on most prediction models in classifying the normal and abnormal levels of fasting glucose and triglyceride cholesterols during baseline and final assessment. Most prediction model recorded accuracy and sensitivity more than 80% with specificity of 50%.

6.2 Limitations

Investigation on supplementation effects usually required longer time of observation to produce more significant and solid proof. However, due to time constraint, the study was designed for 12 weeks or three months assessment for each subject recruited. Recruitment process take most of the time in the study during the data collection phase. In addition, many subjects were reluctant to commit with the three months supplementation thus lengthen the period of the study to reach targeted number of subjects. Besides, in medical analysis more data samples needed to show more conclusive results. The study lacks of data samples that contributed to low performance of the model predictions on MS conditions classification. Despite that, the study achieved the desired outcome which proven that oral glutathione precursors supplementation did produce significant effects in improving the GSH level of its consumer. The prediction model constructed also accurately classify the normal and abnormal level of fasting glucose and triglyceride level based on the GSH level and several other predictors.

6.3 Future works

Through the study, the significant improvement of GSH status recorded after certain time of oral glutathione precursor's supplementation. The metabolic condition of the subjects also showed some changes in relation to the GSH status changes. Observation on patient or individual suffering from specific metabolic disease such as heart disease or diabetic among Malaysians for further study might help in diagnosis of medical problem. Previously, glutathione mostly heard or famously related to cosmetic purposes.

Therefore, with more studies on glutathione relation to metabolic condition might create awareness on people especially women in getting healthy while becoming prettier. Study of glutathione effects on specific range of age subjects also can produce more conclusive results instead of randomized aged subjects. This can establish more precise and accurate effects on the GSH status improvement of certain range of age. Besides that, antioxidant was one of the components in helping to reduce ROS of smokers. Smokers were proven to have depleted glutathione in their blood system. Therefore, effects of smokers after consuming the GSH supplementation also might be one of the interesting topics to be investigate.

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LIST OF PUBLICATIONS AND PAPER PRESENTED

Journal(s)

1. **Nur Rasyidah Hasan Basri**, Mas Sahidayana Mokhtar, Wan Safwani Wan Kamarul Zaman, Geetha Pathmanathan. Predicting Blood Glucose Level in Malaysian Women Based on Glutathione and Anthropometric Parameters. Acta Scientiarum.Technology. (Accepted on 30th October 2020)(Q4).
2. **Nur Rasyidah Hasan Basri**, Mas Sahidayana Mokhtar, Wan Safwani Wan Kamarul Zaman, Geetha Pathmanathan. Glutathione Precursors Supplementation Effects on Renal Function, Lipid Profile and Body Composition (Submitted)

Proceeding(s)

1. **Basri, N. R. H**., Mohktar, M. S., Zaman, W. S. W. K., & Yusof, H. I. M. (2017). The Effects of Oral Glutathione Supplementation on Human Volunteers: An Observation on Metabolic Syndrome Status. IFMBE Proceeding, ICIBEL 2017, Penang. (Published)
2. Asdani Saifullah Dolbashid, Mas Sahidayana Mohktar, Wan Safwani Wan Kamarul Zaman, **Nur Rasyidah Hasan Basri**, Mohd Faiz Azmi, Sakunie Sawai, and Mohd Yusof Hasif Ilyasa. (2017). Effects of Oral Glutathione Precursors' Supplementation on Human Glutathione Level. IFMBE Proceeding, International Conference in Biomedical Engineering and Life Sciences (ICIBEL 2017), Penang. (Published)
3. **Hasan Basri, Nur Rasyidah**, Mohktar, Mas Sahidayana, Wan Kamarul Zaman, Wan Safwani, Azmi, Mohd Faiz, Mohd Syukri, Nursyahirah, Dolbashid, Asdani Saifullah, Zamri, Mohd Izaan Paiz , Mohd Yusof, Hasif Ilyasa (2018). The Effects of Oral Glutathione Differential Dosage on Healthy Overweight Volunteers. Poster

presentation, International Conference on Antioxidant and Degenerative Diseases (ICADD 2018), Kuala Lumpur

4. Wan Kamarul Zaman, Wan Safwani**, Hasan Basri, Nur Rasyidah**, Mohktar, Mas Sahidayana, Azmi, Mohd Faiz, Ahmad Shukri, Nursyahirah, Dolbashid, Asdani Saifullah, Pathmanathan, Geetha (2019). The Effects of Glutathione Precursor's Supplementation on Early Aging Metabolic Syndrome Condition. Poster presentation, International Conference of Ageing (ICA 2019), Penang.