ANALYSIS OF GLUTATHIONE S-TRANSFERASE, PROTEIN TYROSINE PHOSPHATASE 1 B, NUCLEAR FACTOR KAPPA-B1 AND LEPTIN RECEPTOR GENETIC POLYMORPHISMS AS RISK FACTORS FOR TYPE 2 DIABETES MELLITUS IN MALAYSIAN SUBJECTS

ALI ETEMAD

FPSK(p) 2013 7
ANALYSIS OF GLUTATHIONE S-TRANSFERASE, PROTEIN TYROSINE PHOSPHATASE 1 B, NUCLEAR FACTOR KAPPA-B1 AND LEPTIN RECEPTOR GENETIC POLYMORPHISMS AS RISK FACTORS FOR TYPE 2 DIABETES MELLITUS IN MALAYSIAN SUBJECTS

ALI ETEMAD

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA
2013
ANALYSIS OF GLUTATHIONE S-TRANSFERASE, PROTEIN TYROSINE PHOSPHATASE 1 B, NUCLEAR FACTOR KAPPA-B1 AND LEPTIN RECEPTOR GENETIC POLYMORPHISMS AS RISK FACTORS FOR TYPE 2 DIABETES MELLITUS IN MALAYSIAN SUBJECTS

By

ALI ETEMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July/2013
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made within the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
I certify that a Thesis Examination Committee has met on 22 July 2013 to conduct the final examination of Ali Etemad on his Thesis entitled “Analysis of Glutathione S-Transferase, Protein Tyrosine Phosphatase 1B, Nuclear Factor Kappa -B1 and Leptin Receptor Genetic Polymorphisms as Risk Factors For Malaysian Type 2 Diabetes Mellitus Subjects” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

**Fauziah Othman, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Elizabeth George, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Zainul Amiruddin Zakaria, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Lindsay Brown, PhD**
Professor
Faculty of Biomedical Science
University of Southern Queensland
(External Examiner)

______________________________

**NORITAH OMAR, PhD**
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 19 September 2013
DEDICATIONS

My Soul mate Marsa,

My Beloved Mother,

And also to

My supportive brother whom I feel blessed and grateful that I can share this joy with him today. No words can adequately convey the incredible gratitude that I feel for him who was so supportive through this journey
ABSTRACT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ANALYSIS OF GLUTATHIONE S-TRANSFERASE, PROTEIN TYROSINE PHOSPHATASE 1 B, NUCLEAR FACTOR KAPPA-B1 AND LEPTIN RECEPTOR GENETIC POLYMORPHISMS AS RISK FACTORS FOR TYPE 2 DIABETES MELLITUS IN MALAYSIAN SUBJECTS

By

ALI ETEMAD

July/2013

Chairman: Patimah Ismail, PhD

Faculty: Medicine and Health Sciences

Type Two Diabetes Mellitus (T2DM) is one of the serious chronic diseases which are associated with Cardiovascular Disease (CVD) and its complexity though; make it as one of the main mortality contributing factors. The deceive factors such as age, gender, ethnics, lifestyle, genetic backgrounds and their combinations with the environment play an important role in the development of T2DM. The International Diabetes Federation (IDF) predicted the portion of people with Diabetes Mellitus in the world would rise from 285 million in 2010 to 439 million in 2030. The prevalence of T2DM among Malaysian adults was 8.3% a decade ago and became 14.9% in 2009; more dramatically, newly diagnosed T2DM was 1.8% and rose to 5.4% at the same time. In 2011, the same
trend were observed where overall diabetes, known diabetes and newly diagnosed diabetes increased to 21.5%, 11% and 10% for respectively.

In the human genome, several genes were reported as functional candidate genes for T2DM and its associated disease such as CVD, which have intense effects on metabolism, oxidative stress, enzymatic activity and inflammatory expression; genetic variation within these molecules could determine the insulin resistance or leptin regulation and directly increased the risk of T2DM and its complications.

The main objective of this study was to determine the association of genetic polymorphisms of Glutathion S-Transferase (GST), Protein Tyrosine Phosphatase 1B (PTP1B), Nuclear Factor Kappa-B1 (NFK-B1) and Leptin Receptor (LEPR) genes in Malaysian T2DM subjects in comparison with healthy individuals. These genes are also known as positional and functional candidate gene which has association with insulin signaling/resistance and modulate its expression followed by altering the inflammation in different tissues. This research was approved by the Ethical Committee of National Heart Institute (IJNEC/05/10 (02)) and Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (JSB_Mac (12)02).

A total of 587 subjects were approached initially; among them 565 volunteers were recruited for this study. Based on the International Diabetes Federation (IDF) criteria, a total of 284T2DM subjects and 281 healthy individuals as control subjects were recruited under this study. The socio-demographic and other details were recorded using the questionnaire. Genomic DNA was extracted from the blood and buccal cells using commercially available kits. Biochemical analyses were done for the lipid profiles in both subjects. Polymerase chain reaction (PCR), Multiplex-PCR, Restriction Fragment
Length Polymorphism PCR-RFLP methods were used to determine the genetic polymorphisms of the respective genes in all the subjects. The PCR products were separated and analyzed by agarose and polyacrylamide gel electrophoresis. Genotypes were confirmed by DNA sequencing and the banding patterns. The statistical analyses were conducted after exclusion of outliers and the normal values were evaluated by general linear model package through the SPSS statistical software.

The overall allele frequency varies from ($P=0.0494$) in PTP1B-Pro303Pro to ($P=1$) in PTP1B-Pro387Leu. The maximum chi-square belongs to GST loci with four polymorphisms ($P=1620.97$) and the minimum ($P=13.3$) observed for IVS6+G82A polymorphism. There was a significant difference between T2DM subjects and healthy individuals in PTP1B-IVS6+G82A polymorphism ($P=0.007$) followed by LEPR-Gln223Agr polymorphism ($P=0.011$). Also, the anthropomorphic values differed significantly ($P\leq0.01$) for age ($P=0.000$), Body Mass Index (BMI) ($P=0.014$) and Waist Hip Ratio (WHR) ($P=0.000$). The fasting plasma glucose ($P=0.000$) and HbA1C ($P=0.000$) was two critical values for diabetes identification which were significantly different between T2DM and control subjects. The Systolic Blood Pressure (SBP) ($P=0.048$), cardiovascular risk ($P=0.014$), Family history of diabetes ($P=0.007$) and blood lipid patterns significantly differed between T2DM subjects and healthy individuals which included High Density Lipoprotein (HDL) ($P=0.000$), Triglyceride (TG) ($P=0.001$) and total Cholesterol ($P=0.003$). However, the LDL levels of T2DM subjects was under control and not significantly different ($P=0.060$) with healthy individuals.

The association studies and their evaluation based on the Pearson correlation values were conducted in three different categories. There was not a significant correlation...
among the selected polymorphisms but for lifestyle patterns, there was a significant and a negative/indirect *Pearson* value for age versus sex and exercise ($r = -0.153$ and $-0.121$) respectively and positive/direct correlation with T2DM symptoms ($r = 0.327$). Also, there was a direct association between the gender and smoking or alcohol consumption ($r = 0.381$ and $0.305$) respectively and negative association with symptoms ($r = -0.113$).

There was a direct correlation between Smoking/Alcohol and BMI/Symptom ($r = 0.290$ and $0.154$) respectively followed by indirect correlation between exercise and diabetes subjects ($r = -0.117$). The association of the blood lipid patterns was evaluated which was direct and positive between cholesterol and LDL, HDL, TG and Chol/HDL ratio ($r = 0.851$, $0.304$, $0.268$ and $0.489$) respectively. But, there was a negative association between HDL and TG and Chol/HDL ratio ($r = -0.244$ and $-0.574$) respectively. There was a direct correlation between FPG and TG and $H_bA_1C$ ($r = 0.283$ and $0.732$) respectively followed by ($r = 0.091$ and $0.226$) for $H_bA_1C$ and Chol/HDL ratio respectively versus TG levels. Finally, there was an indirect correlation between FPG and LDL ($r = -0.103$) followed by direct association between LDL levels and Chol/HLD ratio ($r = 0.563$).

In conclusion, the diabetes risk factors with the impact of PTP1B-IVS6+G82A polymorphism [age ($P = 0.002$), FPG ($P = 0.000$), $H_bA_1C$ ($P = 0.000$), LDL ($P = 0.012$) and family history ($P = 0.010$)] and LEPR-Gln223Agr polymorphism [age ($P = 0.022$), WHR ($P = 0.000$), FPG ($P = 0.000$), ($P = 0.000$), LDL ($P = 0.000$), HDL ($P = 0.000$) Chol ($P = 0.010$) and family history ($P = 0.000$)] were significantly different between the case and control subjects. This observation could help particularly in case of early diagnoses for
the subjects who have the same genotypic pattern and prevent the diabetes and its complications in high risk categories.

The findings from this research indicated that the genetic polymorphisms [IVS6+G82A ($P=0.007$) and Gln223Agr ($P=0.011$)] of genes (PTP1B and LEPR) respectively were significant between T2DM patients and healthy individuals. This information can be considered as risk factors for the development of T2DM in Malaysian subjects. Apart from that, (BMI, WHR, SBP, HDL, TG, Cholesterol, CVD Risk and family history) were also associated between T2DM and control subjects. It is obviously important to create a database for predicting the risk factors in the Malaysian population in early future which needed a comprehensive data included the environmental factors versus the genetic background and the community attitudes with the prediction of probable epigenetic modifications.
ABSTRAK

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

ANALISIS POLIMORFISME GENETIK S-TRANSFERASE, PROTEIN TIROSINA FOSFATASE 1B, FAKTOR NUKLEAR KAPPA B1, DAN GEN RESEPTOR LEPTIN SEBAGAI FAKTOR-FAKTOR RISIKO BAGI DIABETES MELLITUS JENIS 2 DALAM KALANGAN SUBJEK DI MALAYSIA

Oleh

ALI ETEMAD
Julai/2013

Pengerusi: Patimah Ismail, PhD
Fakulti: Perubatan dan Sains Kesihatan

Diabetes Mellitus Jenis 2 (T2DM) dikenali sebagai salah satu penyakit kronik yang serius dan dikaitkan dengan Penyakit Kardiovaskular (CVD). Kerumitan penyakit ini menjadi faktor penyumbang utama kepada kematian. Faktor-faktor yang memperdayakan seperti umur, jantina, etnik, gaya hidup, latar belakang genetik dan kombinasi antara faktor-faktor ini dengan alam sekitar memainkan peranan penting dalam pembentukan T2DM. Persekutuan Diabetes Antarabangsa (IDF) meramalkan penghidap diabetes di dunia akan meningkat daripada 285 juta pada 2010 kepada 439 juta pada 2030. Prevalens T2DM dalam kalangan orang dewasa di Malaysia ialah 8.3%
sederah lalu dan meningkat kepada 14.9% pada tahun 2009. Lebih dramatik, T2DM baru diagnosis adalah 1.8% dan meningkat kepada 5.4% pada masa yang sama.

Dalam genom manusia, beberapa gen telah dilaporkan sebagai gen calon yang berfungsi untuk T2DM dan penyakit yang berkaitan seperti CVD, yang mempunyai kesan yang besar pada metabolisme, tekanan oksidatif, aktiviti enzim dan ekspresi keradangan; variasi genetik dalam molekul ini boleh menentukan rintangan insulin atau regulasi leptin dan terus meningkatkan risiko T2DM dan komplikasinya.

Objektif utama kajian ini adalah untuk menentukan perkaitan di antara polimorfisme pelbagai genetik iaitu Glutathion S-transferase (GST), Protein Tirosina Fosfatase 1 B (PTP1B), Faktor Nuklear Kappa-B1 (NFK-B1) dan gen Reseptor Leptin (LEPR) yang disiasat dalam subjek T2DM Malaysia dan dibandingkan dengan individu yang sihat. Kajian ini telah diluluskan oleh Jawatankuasa Etika Institut Jantung Negara (IJNEC/05/10 (02) dan Jawatankuasa Etika Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia (JSB_Mac (12) 02).

Seramai 587 subjek telah dikenalpasti pada mulanya. Daripada jumlah itu, 565 orang sukarelawan direkrut untuk kajian ini. Berdasarkan kriteria yang ketat, sejumlah 284 subjek T2DM dan 281 individu yang sihat sebagai subjek kawalan telah diambil untuk kajian ini. Butiran sosio-demografi dan lain-lain telah direkodkan menggunakan borang soal selidik. DNA genom telah diekstrak daripada darah dan sel pipi menggunakan kit yang boleh didapati secara komersial. Analisis biokimia telah dilakukan untuk profil lipid dalam kedua-dua kumpulan subjek. Kaedah Reaksi Rantai Polimerase (PCR), Reaksi Rantai Polimerase Multipleks (Multiplex PCR), Polimorfisme Panjang Fragmen Restriksi (PCR-RFLP) telah digunakan untuk menentukan polimorfisme genetik setiap

Frekuensi alel keseluruhan berbeza dari \( P = 0.0494 \) dalam PTP1B-Pro303Pro \( (P = 1) \) di PTP1B-Pro387Leu. Khi-kuasa dua \( (\text{Chi-square}) \) maksimum kepunyan lokus GST dengan empat polimorfisme \( (P = 1620.97) \) dan khi-kuasa dua minimum \( (P = 13.3) \) diperhatikan untuk polimorfisme IVS6. Terdapat perbezaan yang signifikan antara subjek T2DM dan individu yang sihat pada polimorfisme PTP1B-IVS6-G82A \( (P = 0.007) \) diikuti oleh polimorfisme LEPR-Gln223Agr \( (P = 0.011) \). Selain itu, nilai antropomorfik berbeza dengan ketara bagi nisbah umur \( (P = 0.000) \), BMI \( (P = 0.014) \) dan WHR \( (P = 0.000) \). Glukosa plasma puasa \( (P = 0.000) \) dan \( HbA_1C \) \( (P = 0.000) \) adalah dua nilai kritikal untuk mengenal pasti pasti diabetes yang ketara berbeza antara subjek T2DM dan kawalan. Tekanan Darah Sistolik \( (P = 0.048) \), risiko kardiovaskular \( (P = 0.014) \), sejarah keluarga diabetes \( (P = 0.007) \) dan corak lipid darah ketara berbeza antara subjek T2DM dan individu yang sihat yang termasuk HDL \( (P = 0.000) \), TG \( (P = 0.001) \) dan jumlah kolesterol \( (P = 0.003) \).

Kajian hubungan dan penilaian mereka berdasarkan nilai korelasi \textit{Pearson} telah dijalankan dalam tiga kategori yang berbeza. Tidak ada korelasi yang signifikan antara polimorfisme yang dipilih tetapi untuk corak gaya hidup, terdapat korelasi yang signifikan dan nilai Pearson negatif/tidak langsung masing-masing untuk umur berbanding jantina dan senaman \( (P = -0.153 \text{ dan } -0.121) \) dan nilai Pearson positif/langsung dengan gejala T2DM \( (P = 0.327) \). Terdapat juga hubungan langsung
masing-masing antara jantina dan penggunaan rokok atau alkohol ($P$ =0.381 dan 0.305) dan hubungan negatif dengan gejala ($P$= -0.113). Terdapat hubungan langsung masing-masing antara merokok/alkohol dan BMI/Gejala ($P$=0.290dan0.154) diikuti oleh hubungan tidak langsung antara senaman dan subjek diabetes ($P$=-0.117). Perkaitan corak lipid darah telah dinilai menunjukkan hubungan langsung dan positif masing-masing antara kolesterol dan LDL, HDL, TG dan nisbah Chol/HDL ($P$ = 0.851, 0,304, 0,268dan0,489). Tetapi, terdapat perkaitan negatif masing-masing diantara nisbah HDL dan TG, HbA1C dan Chol/HDL ($P$ = -0.244, -0.102 dan -0.574). Terdapat hubungan langsung masing-masing antara FPG dan TG dan HbA1C ($P$ = 0.283 dan 0.732) diikuti oleh masing-masing ($P$=0.226 dan 0.461) HbA1C dan nisbah Chol/HDL berbanding paras TG. Akhir sekali, terdapat hubungan tidak langsung antara FPG dan LDL ($P$=-0.103) diikuti oleh hubungan langsung antara LDL dan paras nisbah Chol/HDL ($P$=0.563).

Kesimpulannya, faktor-faktor risiko diabetes dengan kesan polimorfisme PTP1B-IVS6 [umur ($P$= 0.002), FPG ($P$= 0.000), HbA1C ($P$= 0.000), LDL ($P$ = 0,012) dan sejarah keluarga ($P$=0,010)] dan polimorfisme LEPR-Gln223Agr [umur ($P$= 0.022), WHR ($P$= 0.000), FPG ($P$ = 0.000) ($P$= 0.000), LDL ($P$ = 0.000), HDL ($P$ = 0.000), Chol ($P$ = 0,010) dan sejarah keluarga ($P$ = 0.000)] ketara berbeza antara subjek kes dan kawalan. Pemerhatian ini dapat membantu terutamanya dalam kes diagnosis awal bagi subjek yang mempunyai corak genotip yang sama dan mencegah diabetes dan komplikasinya dalam kategori berisiko tinggi.

Hasil daripada kajian ini menunjukkan bahawa polimorfisme genetik (IVS6-G82A dan Gln223Agr) bagi gen (PTP1B dan LEPR) masing-masing signifikan antara pesakit
T2DM dan individu yang sihat dan boleh dianggap sebagai faktor risiko untuk pembentukan T2DM dalam subjek di Malaysia. Selain itu, (BMI, WHR, SBP, HDL, TG, Kolesterol, Risiko CVD dan sejarah keluarga) juga mempunyai kaitan antara T2DM dan subjek kawalan. Jelas sekali bahawa adalah penting untuk mewujudkan satu pangkalan data untuk meramalkan faktor-faktor risiko di kalangan penduduk Malaysia dalam masa terdekat yang memerlukan data yang komprehensif yang termasuk faktor-faktor alam sekitar dibandingkan dengan latar belakang genetik dan sikap masyarakat dengan ramalan pengubahsuaian kemungkinan epigenetik.
ACKNOWLEDGEMENTS

Beauty and Admire to God, the Omnipotent, Omniscient and Omnipresent, for opening doors of opportunity for me throughout my life and for giving me the strength and health to achieve what I have so far.

First and foremost, my deepest gratitude to my mother and father (God bless them) who advised and supported me emotionally, mentally and financially to the pursuit of higher education and academic excellence, by expressing understanding and consideration towards me. Words cannot express my gratitude for their love, support, and patience that has sustained me during my life and study. What can I say, except thank you and I shall never forget your kindness and sacrifice.

I would like to express my greatest gratitude to my respected supervisor, Prof. Dr. Patimah Ismail as the chairman of my supervisory committee, for her advice and invaluable guidance towards the period of the study, she really does inspire me since I meet her as a smart, talented, professional behaved manager and generous person.

I would like to express my deepest thanks and gratitude to my supervisory crew Assoc. Prof. Dr. Chong Pei Pei and Dr. Ahmad Fazli Abdul Aziz, for their suggestions, guidance and encouragement throughout this study.
Also, I would like to express my deepest thanks and gratitude to my internal Advisor, Dr. Ramachandaran. Vasudevan, as the only post doctorate fellow in our research group for his guidance from the beginning of my study to proper execution of the research and encouragement throughout this study. He was really inspiring me as a hard worker and talented researcher either.

In addition, I would like to express my deepest thanks and gratitude to my external Advisor, Datuk Dr. Ahmad Khairuddin Mohamed Yusof as a member in National Heart Institute Kuala Lumpur, who helped me a lot to collect the samples for my studies. I will never forget his precious time while he encourages the patients with proper commitment and his professional career as a medical doctor.

Moreover, I would also like to extend my thanks to the respected Department of Biomedical sciences and all of the staff members subsequently, respected staff members of the Faculty of Medicine and Health Sciences. Afterward, the respected staff of the School of Graduate studies, Universiti Putra Malaysia for helping me during the course of my study at UPM.

I would like to express my appreciation to all of the volunteer subjects even the patients from IJN or ordinary people in public screening areas who participated in this research and dedicate their precious specimens and valuable time for this research. Also the
respected staff members of the Institute Jantung Negara (IJN) who assist me a lot to
collect the samples particularly, Ema, Azlina Mohd Saleh and Pn. Haslina.

Special thanks to my friends, Iranians, Malaysians, and those from other places, in
particular to Dr. Arash Javanmard, Dr. Mahmoud Danaei, Seyyed Reza Pishva, Tiffany
NG, Nurul Fasihah Zulkifli, Makanko Komara, Dr.Sima Ataollahi Eshkoor, Dr. Nora
Fawzi, Dr. Hussein Almeamar, Nur Ilyana Binti Jafar, Nur Afiqah Binti Mohamad,
Maryam Jamilah Yusoff, Dr. Farzad Heidari, Nooshin Ghodsian, Salma Aamadloo, Polin
Haghverdizadeh, Sajad Jamalpour, Mohammad Arkani, Mohammad Mehdi Mokhtari,
Human Esmaeiliand others for their help and support during my study whom provides the
lab area calm and friendly.
APPROVAL

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Patimah Ismail, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Chong Pei Pei, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

**Ahmad Fazli Abdul Aziz, MD**
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

**Ahmad Khairuddin Mohamed Yusof, MD**
Department of Cardiology
National Heart Institute
(Member)

---

**BUJANG BIN KIM HUAT, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

________________________

ALI ETEMAD

Date: 22/July/2013
# TABEL OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>ACKNOWLEDGEMENTS</td>
<td>xiii</td>
</tr>
<tr>
<td></td>
<td>APPROVAL</td>
<td>xvi</td>
</tr>
<tr>
<td></td>
<td>DECLARATION</td>
<td>xvii</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xxiii</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xxv</td>
</tr>
<tr>
<td></td>
<td>LIST OF APPENDICES</td>
<td>xxvi</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATIONS</td>
<td>xxvii</td>
</tr>
<tr>
<td></td>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.1 Background</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.2 Problem Statement</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1.3 Significance of the Study</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.4 Hypothesis</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.5 Main Objective</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.6 The Specific Objectives of the Study</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1 Diabetes</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1.1 Type 1 Diabetes Mellitus</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1.2 Type 2 Diabetes Mellitus (T2DM)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.1.3 Epidemiology of T2DM</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.1.4 Metabolic Syndrome (MS)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2.2 Genetics of T2DM</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.3 T2DM and Cardiovascular Diseases</td>
<td>13</td>
</tr>
</tbody>
</table>
### 2.4 T2DM and Cholesterol

### 2.5 Risk Factors for T2DM

2.5.1 Body Mass Index (BMI)  
2.5.2 Lipids  
2.5.3 Hypertension  
2.5.4 Smoking  
2.5.5 Alcohol  
2.5.6 Physical Inactivity  
2.5.7 Dietary Pattern  
2.5.8 Other Risk Factors

### 2.6 Genetic Polymorphism

### 2.7 Candidate Genes

### 2.8 Glutathione S-Transferases (GST)

### 2.9 Protein Tyrosine Phosphatase 1B (PTP1B)

2.9.1 Insulin Signaling  
2.9.2 Leptin Signaling

### 2.10 Nuclear FactorKappa-B1 (NFK-B1)

### 2.11 Leptin Receptor (LEPR)

### 2.12 Different Types of Genetic Studies

2.12.1 Genetic Linkage Studies  
2.12.2 Genome Wide Association Studies  
2.12.3 Candidate Gene Studies

### 2.13 DNA Marker Analyses

2.13.1 Genetic Variation  
2.13.2 Effective Number of Alleles ($n_e$)  
2.13.3 Allele Frequency  
2.13.4 Heterozygosity  
2.13.5 Hardy- Weinberg Equilibrium  
2.13.6 Genetic Distance  
2.13.7 $F_{ST}$ Value

### 2.14 Polymerase Chain Reaction (PCR)

2.14.1 PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)  
2.14.2 Multiplex PCR

### 2.15 Agarose Gel Electrophoresis

2.15.1 Polyacrylamide Gel Electrophoresis (PAGE)
2.15.2. Electrophoresis Gel Concentration 44

2.16 Statistical Methods 45

3 MATERIALS AND METHODS 47

3.1 Study Design 47

3.2 Study Ethics 47

3.3 Duration of Study 47

3.4 Sample Size 48

3.5 Questionnaire 48

3.6 Sample Collection 48
  3.6.1 Inclusion and Exclusion Criteria 50
  3.6.2 Sampling Method 51
  3.6.3 Storage and Transfer of Whole Blood Samples 53
  3.6.4 Separation of Plasma 53

3.7 The Clinical Measurements 54
  3.7.1 Blood Pressure 54
  3.7.2 Body Mass Index (BMI) 54
  3.7.3 Blood Glucose Level 54
  3.7.4 Lipid Studies 55

3.8 DNA Extraction 55
  3.8.1 DNA Quality 55
  3.8.2 DNA Quantification 57

3.9 Optimization of PCR 58
  3.9.1 Positive and Negative Controls 58
  3.9.2 Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) 59
  3.9.3 DNA Sequencing 60
  3.9.4 Enzymatic Digestion 60
  3.9.5 Multiplex PCR 61

3.10 Gene Genotyping 61

3.11 Purification of PCR products 61

3.12 Visualizing 62
3.13 DNA Sequencing 65
3.14 Data Validation 65
3.15 Statistical Methods 65

4 RESULTS 67

4.1 Demographic Distributions 67
4.2 DNA Quantification 73
4.3 DNA Qualification 74

4.4 Glutamin S-Transferase (GSTM/GSTT) Polymorphism 75
   4.4.1 PCR Amplification 75
   4.4.2 Genotypic and Allelic Frequency 76
   4.4.3 Biochemical Patterns 76

4.5 Protein Tyrosine Phosphatase 1B (PTP1B) 79

4.6 IVS6+G82A Polymorphism 79
   4.6.1 PCR Amplification and Enzymatic Digestion 79
   4.6.2 Genotypic and Allelic Frequency 80
   4.6.3 Biochemical Patterns 80

4.7 Pro303Pro Polymorphism 83
   4.7.1 PCR Amplification and Enzymatic Digestion 83
   4.7.2 Genotypic and Allelic Frequency 84
   4.7.3 Biochemical Patterns 84

4.8 Pro387Leu Polymorphism 87
   4.8.1 PCR Amplification and Enzymatic Digestion 87
   4.8.2 Genotypic and Allelic Frequency 88
   4.8.3 Biochemical Patterns 88

4.9 Nuclear Factor Kappa-B1 ATTG₁/ATTG₂ Polymorphism 91
   4.9.1 PCR Amplification 91
   4.9.2 Genotypic and Allelic Frequency 91
   4.9.3 Biochemical Patterns 92

4.10 Leptin Receptor - Gln223Agr Polymorphism 95
   4.10.1 PCR Amplification and Enzymatic Digestion 95
   4.10.2 Genotypic and Allelic Frequency 95
   4.10.3 Biochemical Patterns 96
4.11 DNA Sequencing

4.12 DNA Marker Analysis and Genetic Patterns

4.13 The Interaction Between Selected Polymorphism

4.14 The Combination of Life Style and their Association with T2DM

4.15 The Combination of Lipid Profiles with Blood Glucose Level

5 DISCUSSION

5.1 Internal Risk Factors

5.2 Demographic Risk Factors

5.3 Genetic Studies

5.4 Glutamin S-Transferase

5.5 Protein Thyrosine Phosphatase 1 B

5.6 Nuclear Factor Kappa-B1

5.7 Leptin Receptor

5.8 Genetic Variability among Three Malaysian Ethnics

6 SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

REFERENCES

APPENDICES

BIODATA OF STUDENT

LIST OF PUBLICATIONS