UNIVERSITI PUTRA MALAYSIA

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

ALI ETEMAD

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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

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:

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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 FCTOR 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 ($P \leq 0.01$) 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 H_bA_{1C} (*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%

sedekad lalu dan meningkat kepada 14.9% pada tahun 2009. Lebih dramatik, T2DM baru didiagnosis 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

gendalam semua subjek. Produk PCR telah diasingkan dan dianalisis oleh elektroforesis gel agaros dan polyacrylamide. Genotip telah disahkan oleh penjujukan DNA dan corak jalur. Analisis statistik telah dijalankan selepas pengecualian terpencil dan nilai normal dinilai oleh pakej model linear umum melalui perisian statistik.

Frekuensi alel keseluruhan berbeza dari (*P* =0.0494) dalam PTP1B-Pro303Pro (*P*= 1) di PTP1B-Pro387Leu. Khi-kuasa dua (*Chi-square*)maksimum kepunyaan 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 H_bA_{1C} (*P*= 0.000) adalah dua nilai kritikal untuk mengenal 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 *Pearson* telah dijalankan dalam tiga kategori yang berbeza. Tidak ada kolerasi 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 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, H_bA_{1C} 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), H_bA_{1C} (*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.

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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:

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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

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