



Association of Glutathione S-transferase M1 Polymorphism with Retinopathy in Patient with Type II Diabetes Mellitus

Thesis

Submitted for fulfillment of MSc. Degree in Chemical and Clinical Pathology

Presented by

Radwa Marwan Abd El Halim

M.B.B.Ch, Faculty of Medicine, Cairo University

Supervisors

Prof. Dr. Fatma Ahmed El Mougy

Professor of Clinical and Chemical Pathology

Faculty of Medicine, Cairo University

Dr. Amal Abd El Wahab Mohamed

Professor of Clinical and Chemical Pathology

Faculty of Medicine, Cairo University

Dr. Enas Hamdy Mahmoud

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine, Cairo University

Faculty of Medicine, Cairo University

2012

Acknowledgment

First and foremost, I am always indebted to “ALLAH” the kindest and the most merciful.

*I would like to express my sincere gratitude to **Dr. Fatma Ahmed El Mougny**, Professor of Clinical & Chemical Pathology, Faculty of Medicine, Cairo University, for her continuous guidance and supervision in criticizing and correcting the whole thesis and encouragement which he always offered willingly. To her I extend my sincere thanks.*

*I extend my sincere thanks to **Dr. Amal Abd El Wahab**, Professor of Clinical & Chemical Pathology, Faculty of Medicine, Cairo University, for indispensable help throughout this work.*

*I would like to express my deep gratitude to **Dr. Enas Hamdy Mahmoud**, Assistant Professor of Clinical & Chemical Pathology, Faculty of Medicine, Cairo University, for her continuous help, active support and guidance throughout every step in this work which without her advices this work would not be completed.*

I would like to thank all my staff members and all my colleagues in the Clinical and Chemical Pathology Department, faculty of medicine, Cairo University.

*Of course I am indebted to the **patients** and the volunteers who accepted to be a part of this study. I sincerely hope that this work would provide benefit for them.*

*Special thanks to **my family**; my mother, father and my husband. I dedicate this work to them. They were always supporting and encouraging me to accomplish this work. No words can express my gratitude for them.*

This work was sponsored by a grant awarded from Cairo University, Egypt.

Radwa Marwan

Abstract

Oxidative stress is suggestive to contribute to pathological process in many diseases e.g. cancers, atherosclerosis and diabetes.

Diabetes mellitus is a disorder characterized by hyperglycemia due to an absolute or relative deficiency of insulin and or insulin resistance. Long-term exposure to oxidative stress is strongly implicated in the pathogenesis of diabetic retinopathy. Polymorphic genes of detoxifying enzymes are implied in the development of DR. Homozygous gene deletion of GSTM-1 result in lack of enzyme activity. This study describes that genetic variation in GSTM-1 gene may influence susceptibility to retinopathy in patients with type II diabetes mellitus.

Key words: Diabetic retinopathy - glutathione S-transferase M1

List of Abbreviations

ADA	: American Diabetes Association
ABCC8	: ATP-Binding Cassette transporter subfamily C member 8
AGEs	: Advanced Glycation End Products
AR	: Aldose Reductase
BMI	: Body Mass Index
C-PCR	: Conventional Polymerase Chain Reaction
CDC	: Centers for Disease and Control
DM	: Diabetes Mellitus
DNR	: Diabetic with no retinopathy
DR	: Diabetic retinopathy
FLAP	: 5-Lipoxygenase Activating Protein
GDM	: Gestational Diabetes Mellitus
GSH	: Glutathione
GSSG	: Glutathione Disulfide
GST	: Glutathione transferases
HCC	: Hepatocellular carcinoma
HNF4A	: Hepatic Nucleotide Factor 4 alpha
HNSCC	: Squamous-cell carcinoma of the head and neck
HuGE	: Human Genome Epidemiological Review
INSR	: Insulin Receptor
IR	: Insulin Resistance
LTC4S	: Leukotriene C4 synthase
MAPEG	: Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism
MCP	: Monocyte Chemotactic Protein
NPDR	: Non Proliferative Diabetic Retinopathy
OGTT	: Oral Glucose Tolerance Test
PAH	: Polycyclic Aromatic Hydrocarbons
PDR	: Proliferative Diabetic Retinopathy
PGES1	: Prostaglandin E Synthase 1
PIK3R1	: Phosphatidylinositol 3-Kinase
PPARG	: Peroxisome Proliferator Activated Receptor Gamma

List of Abbreviations

R-PCR	: Real-time PCR
RNS	: Reactive Nitrogen Species
ROS	: Reactive Oxygen Species
SLC2A2	: Solute Carrier 2A 2
TCF7L2	: Transcription cell factor like 2
TNF- α	: Tumor necrosis factor alpha
VEGF	: Vascular Endothelial Growth Factor
WHO	: World Health Organization
MGST	: Microsomal glutathione S transferase
TSO	: Trans-stilbene oxide

List of Tables

	Page
Table 1: Categories of Increased Risk for Diabetes Mellitus.....	5
Table 2: Diagnostic Criteria of Diabetes Mellitus.....	6
Table 3: Classes of Glutathione S Transferase Enzyme.....	34
Table 4: Primers and Probe of GSTM1.....	59
Table 5: Primers and Probe of Cyclophilin.....	59
Table 6: Different Volumes of Reaction Mix.....	60
Table 7: Thermal Cycling Condition for Probe Optimization.....	61
Table 8: Clinical Data of Diabetic Groups.....	64
Table 9: Laboratory Workup of the Studied Groups.....	65
Table 10: Frequency of GSTM-1 Genotypes between the Three Studied Groups....	67
Table 11: Frequency of GSTM-1 Gene Polymorphism in Diabetic Patients with Retinopathy and Control Group.....	67
Table 12: Frequency of GSTM-1 Genotypes in Diabetic Patients with Retinopathy and Diabetic with No Retinopathy.....	68
Table 13: Frequency of GSTM-1 Genotypes in Diabetic Patients with No Retinopathy and Control Group.....	69
Table 14: Frequency of GSTM-1 Gene Polymorphism in Diabetic Patients (DNR & DR) and Control Group.....	70
Table 15: Genotype Frequency of GSTM-1 Gene Regarding Type of Retinopathy (PDR\NPDR).....	71
Table 16: Frequency of Lipid Profile in Different GSTM1 Genotypes among Diabetic Cases.....	73
Table 17: Comparison of Lipid Profile in GSTM1 genotype among Diabetic Patients.....	73
Table 18: Clinical and Laboratory Data of the Diabetic Group with No Retinopathy.....	76
Table 19: Clinical and Laboratory Data of the Diabetic Group with Retinopathy.....	78
Table 20: Clinical and Laboratory Data of the Control Group.....	79

List of Figures

	Page
Figure 1: Obesity induces alterations in adipose tissue, liver, and skeletal muscle that result in systemic insulin resistance and inflammation	11
Figure 2: Factors regulating macrophage polarity and insulin resistance in adipose tissue	14
Figure 3: Biological function of T2DM candidate genes arising from animal models and human association studies.....	17
Figure 4: Mechanism of insulin release.....	19
Figure 5: Pathways to Type II Diabetes Implicated by identified common variant associations	21
Figure 6: Dynamic balance between the biosynthesis of GSH.....	31
Figure 7: Involvement of MAPEG in leukotrienes and prostglandins biosynthetic pathways	36
Figure 8: Structure of the GSTM1 region and location of primers	38
Figure 9 : Role of GST in the elimination of polycyclic aromatic hydrocarbons	42
Figure 10 : Oxidative stress and dysmetabolism in diabetes	49
Figure 11: TaqMan probe based chemistry	58
Figure 12: Real-Time PCR analysis of GSTM1 gene polymorphism	61
Figure 13: The mean values of triglyceride, cholesterol and LDL in the studied groups	65
Figure 14 : The median values of HDL in the studied groups.....	66
Figure 15: Frequency of GSTM-1 gene polymorphism in diabetic patients with retinopathy and control group.....	68
Figure 16: Frequency of GSTM-1 genotypes in diabetic patients with retinopathy and with No retinopathy.....	69
Figure 17: Frequency of GSTM-1 genotype in diabetic patients with No retinopathy and control group.....	70
Figure 18: Frequency of GSTM-1 genotype in diabetic patients (DNR & DR) and control group.....	71
Figure 19: Genotype frequency of GSTM-1 gene regarding type of retinopathy (PDR\NPDR).....	72

List of Figures

- Figure 20:** The median values of triglycerides regarding GSTM1 genotype in diabetic patients with dyslipidemia.....74
- Figure 21:**The mean values of T.cholesterol, HDL and LDL (mg/dl) regarding GSTM1 genotype in diabetic patients with dyslipidemia74
- Figure 22:**The mean values of T.cholesterol/HDL level in different GSTM-1 genotype In diabetic patients with dyslipidemia.....75

Contents

	Page
Introduction and Aim of the work	
Review of Literature.....	1
Subjects and Method.....	52
Results.....	63
Discussion.....	80
Summary and Conclusion	87
Recommendations.....	89
References.....	90

الملخص العربي

Introduction and Aim of the Work

Introduction and Aim of the Work

Introduction

Glutathione S-transferases (GSTs) consist of a superfamily of dimeric phase 2 metabolic enzymes that catalyse the conjugation of glutathione with wide range of electrophilic substrates (*Habdous et al., 2004*).

These enzymes play an important role in detoxifying cytotoxic agents and protecting cellular macromolecules. Oxidative stress is suggestive to contribute to pathological process in many diseases e.g. cancers, atherosclerosis and diabetes (*Chen et al., 2005 and Hori et al., 2007*). It also plays an important role in the development of microvascular complications in type II diabetes (*Wang et al., 2006 and Hossaini et al., 2008*).

At least seven distinct classes of soluble GST that are expressed in mammalian liver have been identified: alpha, mu, pi, sigma, kappa, omega and theta.

GST mu (GSTM1) locus has been mapped to chromosome 1 p13.3 and it is considered as one of the most common sites for polymorphism of GST genes in human (*Hovnik et al., 2009*).

Subjects with at least one functional allele for GSTM1 are grouped into the positive conjugator type and called GSTM1 positive.

Homozygous gene deletion of the GSTM1 result in complete lack of enzyme activity with a frequency 40-60% & it is called GSTM1-null (*Landi, 2000*).

It was reported that genetic variation in glutathione S- transferase M1 (GSTM1) gene may influence susceptibility to retinopathy in patients with type II diabetes mellitus.

Aim of the work

The present study aimed to assess the association between GSTM-1 gene polymorphism and the development of retinopathy in type II diabetes mellitus.

CHAPTER 1

Diabetes Mellitus

Diabetes Mellitus

Definition

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or action, or both. Insulin enables cells to absorb glucose in order to turn it into energy. This causes glucose to accumulate in the blood (hyperglycemia), leading to various potential complications *(Rother, 2007)*.

Incidence

Diabetes mellitus has become one of the most challenging health problems of the 21st century. It affects more than 230 million people worldwide, and this number is expected to reach 350 million by 2025. It is the fourth leading cause of death by disease globally; every 10 seconds a person dies from diabetes-related causes *(Kowluru and Chan, 2007)*.

In the United States an estimated 20.8 million people have diabetes. Unfortunately, the disease does not go away, but it can be controlled.

A study conducted by the Centers for Disease Control suggests that diabetes care has improved over the past 10 years; but there remains a great need to focus on additional improvements because about 850 000 new cases of diabetes are diagnosed each year in United States alone *(Sharma et al., 2005)*.